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NEURONAL AND HORMONAL INFLUENCES ON THE LIFE-SPAN OF THE ROTIFER ASPLANCHNA BRIGHTWELLI, GOSSE

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ABSTRACT

NEURONAL AND HORMONAL INFLUENCES ON THE LIFE-SPAN OF THE ROTIFER ASPLANCHNA BRIGHTWELLI, GOSSE

Vera Bozovic

The object of this study was to determine whether the lifespan of the rotifer Asplanchna brightwelli could be influenced via neuroendocrine pathways. Three nerve blockers and four hormones were examined for their effect on lifespan.

Nicotine, atropine and procaine are acetylcholine specific nerve blockers. Nicotine and procaine had no effect on lifespan. In contrast, atropine caused an increase in rotifer lifespan. It was found that the rotifers exposed to atropine ate less than control rotifers. This was observed by monitoring and comparing their ingestion of neutral red stained Paramecia. It is known that dietary restriction increases lifespan in the rotifer. The mechanism by which atropine extends lifespan may be through interference with feeding, thus imposing dietary restriction on the organism.

Groups of rotifers were also exposed to varying concentrations of the following hormones: B-estradiol, thyroxine, cortisone and hydrocortisone.

The hormone B-estradiol was toxic to the rotifers. Thyroxine had no effect on rotifer lifespan. Cortisone and hydrocortisone both increased rotifer lifespan. Cortisone increased the length of the prereproductive and reproductive periods and the number of offspring. While hydrocortisone increased the rotifer's prereproductive period, it caused a decrease in both reproductive period and offspring number. Cortisone and hydrocortisone were believed to affect the lysosomes in the rotifer; histochemical staining indicated a decrease or possibly an alteration in lysosomes in rotifers exposed to cortisone or hydrocortisone. Furthermore, rotifers exposed to these hormones were smaller in size than controls.

A common mechanism by which cortisone and hydrocortisone increase rotifer lifespan may be by decreasing cellular breakdown and altering growth rate.

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INTRODUCTION

The two physiological systems which are most important in integrating the homeostasis, activity and metabolism of any multicellular organism are the nervous system and the endocrine system. For this reason it is thought that both systems must play important roles in the control of lifespan (Adelman & Roth, 1982). Although most research on neuroendocrine control of lifespan has concentrated on vertebrates and particularly on mammals, it is known that invertebrates also have cholinergic neurons (Barrington, 1967, 1975) and hormone receptors (Barrington, 1975).

In this thesis, my work was directed toward answering two experimental questions: (1) What are the effects of cholinergic nerve blockers on rotifer lifespan, activity and feeding? and (2) Can rotifer lifespan and reproduction be influenced by specific hormones?

The theoretical background and rationale for these questions will be examined in the sections of the introduction which follow.

First, the reason why rotifers were advantageous experimental organisms in a study of this type is that they have a short lifespan of 5 to 6 days. Thus many experiments can be accomplished in a very short period. Furthermore, rotifers are easy to maintain (see "Materials and Methods" section). These organisms reproduce by parthenogenesis; rotifers are all females and thus genetic clones. Male rotifers only occur under unusual environmental conditions and are rare. Rotifers have been used succesfully as experimental organisms and aging models in the past (Verdone-Smith, 1981; Sawada, 1983; Beauvais and Enesco, 1985).

The Nerve Blockers

The interest in studying nerve blockers in rotifers was initiated by previous work done by Beauvais and Enesco (1985). In that study the effects of the acetylcholine specific nerve blocker curare on rotifer lifespan and activity level were observed; it was found that low concentrations of curare increased rotifer lifespan and decreased the activity level of the rotifers.

The objective of the present study was to determine if any other acetylcholine specific nerve blockers have similar effects on the rotifer Asplanchna brightwelli.

Experiments done by Nogrady and Alai (1983) have indicated the presence of acetylcholine (ACh) in the rotifer. Nogrady and Alai (1983) have shown the existence of acetylcholinesterase, which causes hydrolysis of ACh to acetate and choline, and choline acetyltransferase, which catalyzes the reaction of acetyl CoA and choline to form ACh in twelve rotifer species.

The neuromuscular endplates, parasympathetic postganglionic synapses and all ganglia in the central nervous system of vertebrates release the neuro -transmitter acetylcholine (Guyton, 1981). In the vertebrates, acetylcholine attaches to and activates two different types of receptors: the nicotinic and muscarinic receptors.

It is known that the invertebrate groups Nematoda, Annelida and Platyhelminthes all posess acetylcholine (Leake & Walker, 1980). As far back as 1935, Pantin examined different animal phyla to determine if cholinergic and adrenergic systems occur in these animals; his conclusion was that both systems were present in all coelomates, coelenterates, sponges and protozoans. Since then there has been both contradictory and confirming research done. Florey (1983) demonstrated that acetylcholine reyokes a response in tunicate muscle

preparations and Futamachi (1972) showed the flexor abdominal muscle of a brachyuran crustacean is depolarized in the presence of acetylcholine; this response was increased by anticholinesterase and blocked by D-tubocurarine. Terada (1982) has found that acetylcholine causes contraction and increased motility in the parasitic helminth Angiostrongylus cantonensis (rat lungworm). It was assumed that the excitatory cholinergic mechanism in A. cantonensis was nicotinic because only nicotine agonists evoked a response. Lentz and Barrnett (1962) discovered ACh in the nematocyst discharge of Hydra litorallis, indicating the presence of ACh in hydrozoans.

As mentioned previosly, curare, a muscarinic nerve blocker, extended rotifer lifespan and decreased activity level. It is suspected from the these results that the rotifer has muscaric receptors; the goal of this study was to confirm this suspicion and also to determine whether the rotifer has nicotinic receptors. The results of the curare experiment were interpreted in relation to the "rate of living" theory of aging, which states that the slower the metabolism of an organism, the longer it lives. It was of interest to examine if the use of other nerve blockers would support the same theory.

In vertebrates, muscarinic receptors are present in the motor neurons which are stimulated by the postganglionic cholinergic neurons of the sympathetic system and these receptors also occur in all the motor neurons stimulated by the postganglionic nerves of the parasympathetic system in vertebrates (Guyton, 1981). The synapses between the pre-and postganglionic neurons of both the sympathetic and parasympathetic systems contain the nicotinic receptors, as do the membranes of skeletal muscle fibers at the neuromuscular junction (Guyton, 1981).

The method used to block acetylcholine action is to introduce ACh specific blockers; these nerve blockers attach to ACh receptors and they do not allow ACh to attach to and activate these receptors. There are blockers specific for nicotinic and muscarinic receptors respectively. In this study, three different nerve blockers were used; atropine (muscarinic), nicotine (nicotinic) and procaine (nicotinic). These nerve blockers have been found to affect invertebrates other than the rotifer.

For example, Yang (1983) determined that when procaine was applied to specific leech neurons, the maximum rates of depolarization and repolarization during

action potentials was decreased. Apparently procaine acted by blocking sodium permeability during excitation of the neuron. Addition of nicotine to body wall strips and oral rings of the sea anemone Buonodosoma caissarum caused contraction of the tissues (Mendes, 1984). Hillman (1983) did activity studies on the Nematoda Brugia malayi using various anticholinergic drugs, one being atropine. The atropine was added to the medium in which the worms were located and their activity monitored via an activity monitor. Results showed that atropine significantly reduced motor activity in the worm.

These findings therefore suggest that invertebrates do have muscarinic and/or nicotinic receptors, since they respond to cholinergic nerve blockers.

The Hormones

The importance and function of hormones in mammalian systems is well established (Guyton, 1981). Hormone receptors have recently been observed in microorganisms and in invertebrates (Martin and Spencer, 1983; Barrington, 1967, 1975). There is reason to believe that these hormones originated early in evolution to serve some kind of signalling or integrating system.

The role of hormones in invertebrates is not clear.

Nevertheless, we examined the following question: Could hormones effect the lifespan and reproduction of the rotifer? The hormones used in this study were the thyroid hormone, thyroxine, the reproductive hormone, B-estradiol and the glucocorticoid hormones, cortisone and hydrocortisone.

In mammals, thyroxine is stored in the follicles of the thyroid gland and is synthesized by iodides (Guyton, 1981). Thyroxine increases metabolic rate, muscle movement (in excess, causes erratic and vigorous tremours) and increases general nervous action in mammals (Guyton, 1981).

The rotifer posesses both smooth and striated muscle fibers, visceral and cutaneous muscles to hold the internal organs in place, as well as a primitive nervous system consisting of a sac-shaped cerebral ganglion (brain) and some nerve fibers and nerve cell bodies innervating the sensory areas, muscles and viscera (Pennak, 1978). Thus, it was of interest to observe if thyroxine had any effect on the muscular system and/or nervous system of the rotifer. Thyroxine receptors have been detected in lower organisms; Csaba (1980) found that the thyroxine precursors mono- and diiodothyrosine increased the growth rate of the protozoa Tetrahymena

pyriformis, suggesting the presence of thyroxine specific hormone receptors in this organism.

In organisms with known estrogens, the estrogens are secreted by the ovaries (Guyton, 1981). Estrogens are responsible in vertebrates for development of secondary sexual characteristics (sexual maturation) of female organisms (Guyton, 1981). These hormones are all steroids and are synthesized from cholesterol or acetyl coenzyme A. The most potent of the estrogens is B-estradiol.

Invertebrates have been found to posess sex hormones (Barrington, 1975). Crustaceans, for instance, have an ovarian hormone, which is responsible for oocyte development and secondary sexual characteristics in the female which include a brood pouch (Barrington, 1975).

Rotifers are much more primitive than mammals, but these organisms do reproduce by parthenogenesis, have a vitellarium and could theoretically possess some type of reproductive hormones. Even in a primitive organism such as the rotifer, this hormone might affect lifespan and reproduction.

Cortisone and hydrocortisone are called glucocorticoids because they have a significant influence in increasing glucose concentration, yet they also reduce

protein levels in body cells and mobilize fatty acids from adipose tissue in mammals (Guyton, 1981). It has been suggested that insufficient levels of these hormones leads to fragile lysosomes in skeletal muscle (Hardy, 1981). These hormones are known to have anti-inflammatory action which they exhibit most effectively by stabilizing lysosomal membranes (eliminate rupturing) (Symons, 1969; Pollock, 1971). Lysosomes contain many degradative enzymes which are extremely harmful to the cell if released (Dean, 1977). During the aging process, the lysosomes become more fragile and break, causing release of the lytic enzymes which can destroy the cell by digesting the cell contents (Dean, 1977).

Since the rotifer has lysosomes, it seemed useful to examine if cortisone and hydrocortisone had any effect on lysosomes within the rotifer in conjunction with rotifer lifespan and reproduction.

Cortisone and hydrocortisone effect metabolic processes, such as reducing protein metabolism and increasing lipid metabolism. In mammals, the decreased protein metabolism has various effects such as suppression of growth in young animals (less bone growth) decreased collagen in skin and decreased growth and replication in fibroblasts (Tepperman, 1981).

These facts gave rise to the question of whether rotifer metabolism or growth could be affected by cortisone and hydrocortisone.

Objectives

This study consists of two separate parts and deals with neuronal and endocrine factors which might influence lifespan in the rotifer Asplanchna brightwelli.

The experiments that will be described in the first part of this study examine the effects of acetylcholine nerve blockers on the rotifer. The objective is to determine if the nerve blockers, nicotine, atropine and procaine affect the rotifer's lifespan and reproductive profile. If changes are observed, appropriate experiments will be done to determine how and why lifespan and fecundity were affected.

The experiments carried out in the second part of this study will examine the effects of hormones on the rotifer. The objective is to ascertain if the hormones, thyroxine, B-estradiol, cortisone and hydrocortisone affect the rotifer's lifespan and reproduction. Any changes that occur will be examined to determine how and why lifespan and fecundity were affected.

Materials and Methods

The Rotifer:

The rotifers used were of the species Asplanchna brightwelli, Clone 4B61; these organisms were originally obtained from Dr. John J. Gilbert, Dartmouth College, Hanover, New Hampshire. These rotifers have a short lifespan of 5 to 6 days and since they reproduce by amictic parthenogenesis, they are all females and genetically identical (clones).

Culture Methods:

The rotifers were maintained along with their food source, Paramecium caudatum, in a Cerophyll infusion medium. The medium consisted of 2000 ml distilled water, 0.12 g $CaCO_3$ and 14 ml of stock buffer solution in a 4-liter Erlenmeyer flask; the mixture was heated to a boil. Once the solution was boiling, 1.5 g of cerophyll (Ward's Natural Science Establishment, Rochester, New York) was added and the entire solution was boiled for 10 minutes. The solution was cooled on ice to room temperature and then filtered through Whatman no.3 filter paper. The

solution was autoclaved for 20 minutes and then cooled under running tap water.

A loopful of E.coli (Type K12, originally obtained from Dr. Newman, Concordia University) was added to the medium which was then incubated at 37° C for 24 hours. After this time the pH of the medium was measured to ensure that it was between 6.5-7.0. A 0.05 N NaOH solution was added if the pH needed to be adjusted; but this was usually unnecessary. Fifty milliliters of old medium containing Paramecium caudatum (originally obtained from Boreal Laboratories Ltd., Mississauga, Ontario) was added to the fresh solution. The paramecium were left to multiply for two to three days (36-72 hours). After this time, 150 ml of the medium was added to a 250 ml Erlenmeyer flask and 20 rotifers of various ages were transferred to the flask; 5 of these "stock culture" flasks were kept at room temperature and every 5 days the rotifer stock was replenished with fresh paramecium medium.

Approximately 300 ml of the paramecium medium was kept aside to be used for freshly prepared medium, which was made every 10 days.

Experimental Methods:

Each experiment began with freshly prepared paramecium medium, stock buffer and E.coli cultures.

Every experiment was initiated by placing rotifers from the stock cultures in a Petri dish and examining them under a dissecting microscope. Adult rotifers were removed and individually plated in a tissue cluster dish with 24 wells (No. 76-063-05, Linbo brand, Flow Laboratories, McLean, Va.). A micropipette (Finnipipette, Ky, Helsinki, Finland) was used to transfer the rotifers. Prior to adding the rotifers to the wells, 2.5 ml of paramecium medium was added to each well.

The individual wells were examined for offspring every three hours. Only newborn rotifers (0 to 3 hours old) were removed and used for the experiments, since their age was established.

Lifespan Experiments:

Specific nerve blockers and hormones were used to observe if they effected rotifer lifespan.

Newborn rotifers were placed in individual wells of the tissue cluster dish. Prior to adding one rotifer per

well, 2.5 ml of the required solution (control medium or medium containing the chemical) was added to each well. Twenty-four rotifers were used for each control and each of the samples (different concentration of the various nerve blockers and hormones). The rotifers were fed once a day; 1 ml of the solution in the well was replaced with a fresh identical solution.

The control and rotifers exposed to the various concentrations of nerve blockers and hormones were checked every six hours for offspring and to observe if they were still alive; the offspring were counted and immediately discarded. The rotifer was regarded dead when its cilia ceased to move or when it showed signs of deterioration.

Temperature:

For all of the experiments, the rotifers were maintained in an incubator at 19 ± 0.5 ^{o}C, since it had been previously determined that the mean rotifer lifespan was longest at this temperature (Sawada, 1983) and the room temperature was between 19 - 20 ^{o}C.

The Nerve Blockers:

For each experiment there was a control; one

control for each nerve blocker used and this control consisted of regular paramecium medium which contained no nerve blocker.

The nerve blockers used were nicotine (Sigma Chemical Co., St. Louis, Missouri), atropine (Sigma Chemical Co.) and procaine (Laboratories Winthrop, Aurora, Ontario). The stock solutions of the nerve blockers were 1% nicotine stock, 0.1% atropine stock and 0.1% procaine stock. Various amounts of the stock solutions were added to the regular paramecium medium to obtain the desired concentrations of the nerve blockers. The concentrations that were used are as follows:

a. nicotine concentrations: 0.000001%, 0.0000025%, 0.000005%.

b. atropine concnetrations: 0.0001%, 0.00025%, 0.0005%

c. procaine concentrations: 0.00001%, 0.000025%, 0.00005%, 0.0001%, 0.00025%, 0.0005%.

The lifespan experiments were done four times for each individual nerve blocker used.

Movement Experiments:

When a certain nerve blocker produced a lifespan increase in the rotifer, this blocker was examined for

possible effects on activity by observing how fast the rotifer moved.

While monitoring and comparing the movement of the controls to the nerve blocker-treated rotifers, the rotifers remained in the individual 2.5 ml wells of the tissue culture dish. A transparent plastic sheet was placed underneath the well; a 1 cm grid with individual squares of 1 mm each had been drawn on the sheet. The number of 1 mm squares in the grid that the rotifer transversed in one minute were counted.

The Hormones:

For each experiment there was a control; the control consisted of regular paramecium medium which contained no hormone. Since the hormones had to be solubilized in ethanol, an ethanol control was also run with each experiment; this control consisted of the same concentrations as the hormones.

The hormones used were thyroxine (Sigma Chemical Co., St. Louis, Missouri), B-estradiol (Sigma Chemical Co.), cortisone (Sigma Chemical Co.) and hydrocortisone (Sigma Chemical Co.). The stock solutions of the hormones were 0.1 M thyroxine stock, 0.1 M cortisone stock, 0.1 M B-estradiol stock and 0.01 M hydrocortisone

stock. These stock solutions were made up in 100 % ethanol. Various amounts of the stock solutions were added to the regular paramecium medium to obtain the desired concentrations of the hormones.

The concentrations were the same for each of the hormones used and are as follows:
10 uM, 50 uM, 100 uM, 200 uM, 400 uM, 800 uM.
These concentrations were also used for the ethanol control.

Every lifespan experiment was done four times for each hormone respectively.

Staining Procedures:

- Neutral Red

This stain was made by first preparing a stock solution of 1 mg/ml neutral red chloride (lot No. 021387; C.I. No. 50040, Aldrich Chemical, Milwaukee, Wisconsin) with distilled water. A neutral red concentration of 0.75 ug/ml was prepared from the stock solution and the culture medium. This concentration had been previously found to be most effective in paramecium coloration (Sawada, 1983).

The paramecium within the culture medium were exposed to the neutral red for eight hours to allow

uptake of the dye by the organism. After eight hours, a 0.0001% and 0.00025% atropine solution were prepared using the atropine stock solution and the paramecium containing 0.75 ug/ml neutral red solution. The final solutions were added to tissue cluster dishes and then rotifers were placed individually in the wells. The rotifers were exposed to the dye for two days and then observed for staining of the stomach; an indication of ingestion of paramecium which was observed as a dark red stain within the rotifer. Control rotifers were also exposed to the same concentration of neutral red solution.

The darkness of the gut coloration in comparison to the control would indicate whether fewer paramecium had been consumed by the atropine-treated rotifers.

Since newborn Asplanchna do not eat for several hours (Birky, 1964), the rotifers used for the neutral red experiments were at least 12 hours old.

- FluoraBora·I (FBI)

This fluorescent vital stain, which is specific for lysosomes, is prepared with a specific buffer called 3-(N-morpholino)-2-hydroxypropanesulfonic acid (MOPSO) (No. 8760; Polysciences Inc., Warrington, PA.). The FBI (m-

dansylamido-phenylboronic acid) (No. 16836; Polysciences Inc, Warrington, PA) solution was prepared in 25 mM MOPSO containing 140 mM NaCl and adjusted to pH 7.4 with NaOH (Gallop, 1982).

The process by which this stain works is by transferring water-insoluble agents across cell membranes and is referred to as "Boradeption". In this process, highly fluorescent boronic acid derivatives, such as the fluorophores (FluoroBoras), are solubilized with a carrier buffer which is physiologically compatible. The buffer contains a receptor for boronate adduct formation and this system stains living cells.

Certain reporter groups such as the FluoroBoras, are coupled to boronic acids to form ReportaBoras. A 1,3-diaryl-pyrazoline (an optical brightener), 1-(phenyl-p-sulfonic acid), 3-phenyl-pyrazoline sulfonyl chloride is coupled to m-aminophenylboronic acid (Gallop, Paz and Henson, 1982). Eventhough the fluorescent pyrazoline-sulfamido-phenylboronic acid is highly insoluble in water, once it is added to specific carrying buffers that form complexes with boronic acids, it can be solubilized at physiological pH. Once solubilized, the entire complex can be carried into an aqueous solution and the free compound can move through the hydrophobic areas on the

cell membranes and finally entering cellular components, such as lysosomes.

Lysosome Accumulation:

Three categories of rotifers were exposed to the FBI stain; rotifers exposed to cortisone and hydrocortisone solutions respectively and control rotifers (no hormone).

The concentrations of cortisone used were 10, 50, 100 and 200 uM, the concentrations of hydrocortisone were 10, 50 and 100 uM.

At each of the concentrations for each of the hormones and the controls, rotifers from one to six days old were stained and examined for presence of lysosomes in terms of staining intensity.

Microscopy:

To observe the rotifers stained with the FBI stain, they were removed from the wells of the culture dish. Rotifers were examined for presence of fluorescence from day 1 to day 6. The rotifers were transferred to a clean concave slide with 20 ul of medium via a micropipette. Twenty microliters of the vital stain was then added to the rotifer on the slide. After 5 minutes the rotifers were examined under a Zeiss Ultraphot II Phase Contrast Microscope at a magnification of 40X.

Photography:

The rotifers stained with neutral red were photographed under a C. Reichert (Nr.309060) light microscope which had a camera attachment. An 10X ocular with a Plan 4/0.10 objective was used resulting in a final magnification of 10X. Kodacolor VR color film, ASA 1000 was used. The rotifers were fixed in 50% ethanol for 3 minutes before being photographed. The ethanol immobilized the rotifers and caused a slight size enlargement.

Size Measurements:

The rotifers were washed twice in distilled water to remove the paramecium and then added to 0.1 mg/ml neutral red solution for 5 minutes. This solution did not kill the rotifers but only immobilized them so that their body size could be measured.

The microscope used was an Olympus. To measure the rotifer's body size a calibrated 6X ocular micrometer was used with a 4X objective, resulting in a final magnification of 24X.

Statistical Analysis:

A one-way analysis of variance followed by a post

hoc Tukey test was carried out to determine the statistical significance of the data obtained from all the studies done (Brunning & Kintz, 1977; Sokal & Rohlf, 1981; Zar, 1984).

RESULTS

During each of the initial experiments, it was necessary to determine the normal lifespan and fecundity of *A. brightwelli*. That is to say that the lifespan and fecundity of control untreated rotifers were compared with the lifespan and fecundity of rotifers treated with a certain concentration of hormones or nerve blockers.

Each of the initial lifespan experiment was repeated four times with each chemical respectively. Only one one set of results for each chemical is described in detail in this section. The averages and F values for the replicate experiments are presented in Appendix II.

The lifespan of the rotifer was divided into three developmental stages; prereproductive, reproductive, and postreproductive periods. These stages were collectively referred to as the reproductive profile. The chemicals used could thus be examined for their effect on any or all of the different stages of development.

The Nerve Blockers

Determination of Optimum Nerve Blocker Concentration

The purpose of the experiments carried out in this section was to examine if and how various concentrations of the different nerve blockers would modify life-span and fecundity.

The nerve blockers used were nicotine, procaine and atropine. These compounds are water soluble, so that they could be dissolved directly in the medium. The control rotifers were placed in *Paramecium* medium alone.

Nicotine

Longevity

Table 1 displays the longevity data for rotifers exposed to nicotine concentrations of 0.000001, 0.0000025 and 0.000005 % and as compared to the control. As can be seen from the table, the rotifer lifespan is not affected by any of the nicotine concentrations. A one-way analysis of variance showed that there were no significant differences in lifespan between the control group and the rotifers exposed to the different nicotine

concentrations ((F 3, 92) = 0.661, p>0.05).

The relationship between the lifespan values can be further emphasized graphically as in Figures 1 and 2. Figure 1 consists of survival curves of control and nicotine groups and shows that the curves all lie within the same area. Figure 2 shows a lifespan versus concentration graph and reveals the relationship between lifespan and increasing nicotine concentrations. This graph indicates that there is a slight increase in lifespan for nicotine-treated groups in comparison to the control, but it is not very great; the lifespan values all fall within a very close range (5.3 -5.72).

The reproductive profile, incorporating prereproductive, reproductive and postreproductive period, of the control and the different nicotine concentrations is displayed in Table 2. The one-analysis of variance followed by the post hoc Tukey test revealed no significant difference between any groups at any of the stages of the reproductive profile.

Table 1

THE EFFECT OF NICOTINE ON THE LIFE-SPAN OF A. BRIGHTWELLI
(N = 24)

Concentration of Nicotine solution (percent)	Maximum Longevity (Days)	Mean Lifespan ± S.E.M. (Days)
0	7.0	5.40 ± 0.27
0.000001	6.0	5.30 ± 0.27
0.0000025	7.0	5.72 ± 0.20
0.000005	6.0	5.64 ± 0.22

-No significant difference between any group

FIGURE 1. Survivorship curves of *A. brightwelli* exposed to different nicotine concentrations as compared to control. (n = 24)

control = 0 nicotine concentration

$1.0 * 10^{-6}$ % = 0.000001 % nicotine concentration

$2.5 * 10^{-6}$ % = 0.0000025 % nicotine concentration

$5.0 * 10^{-6}$ % = 0.000005 % nicotine concentration

% = percent

n_x = number of survivors at start of age interval x

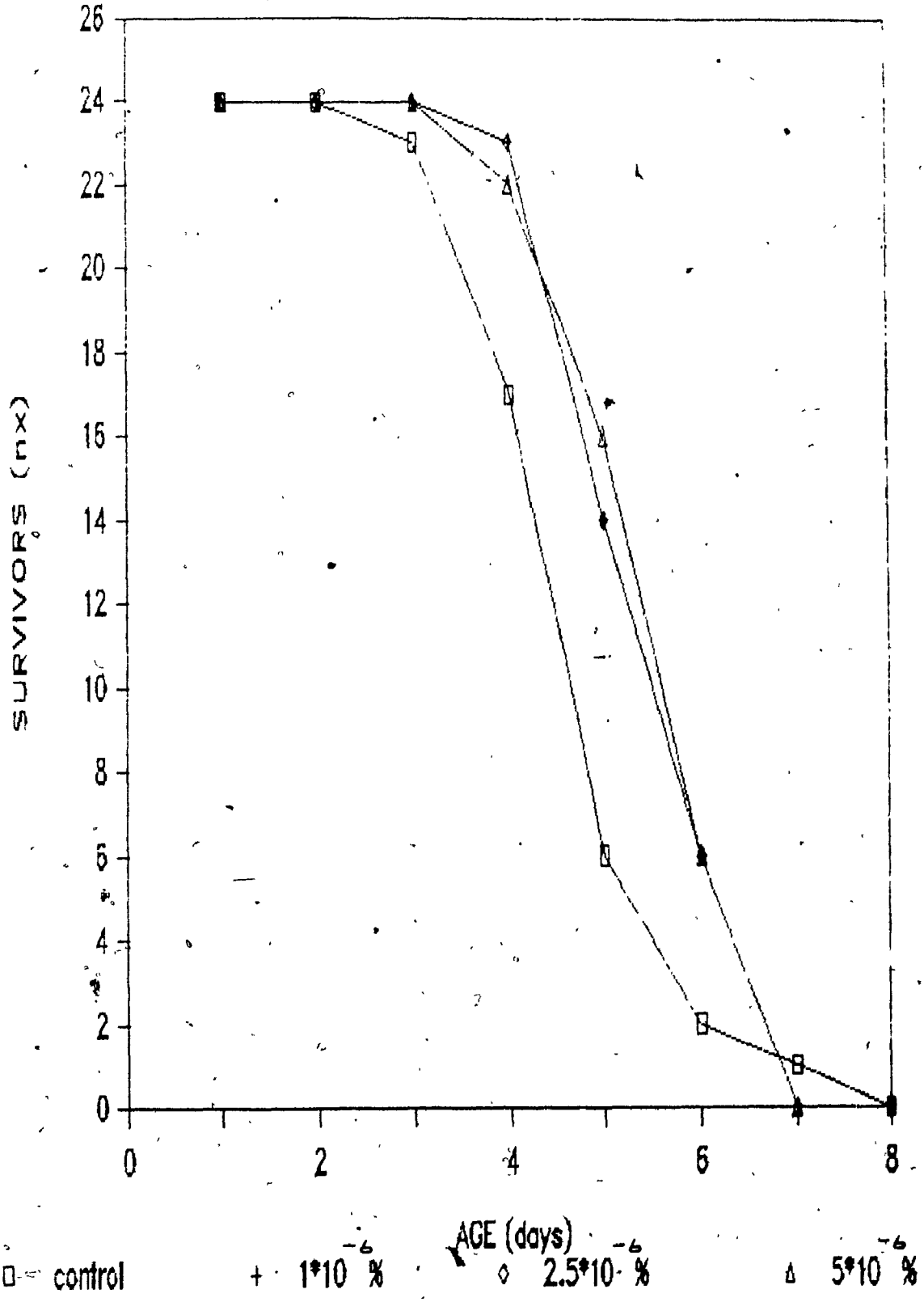

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
□ control
+ 1*10 -6 %
◇ 2.5*10 -6 %
△ 5*10 -6 %

FIGURE 2. Life-span versus nicotine concentration graph of A. brightwelli exposed to different nicotine concentrations. (n = 24)

E-06 percent = 10^{-6} %

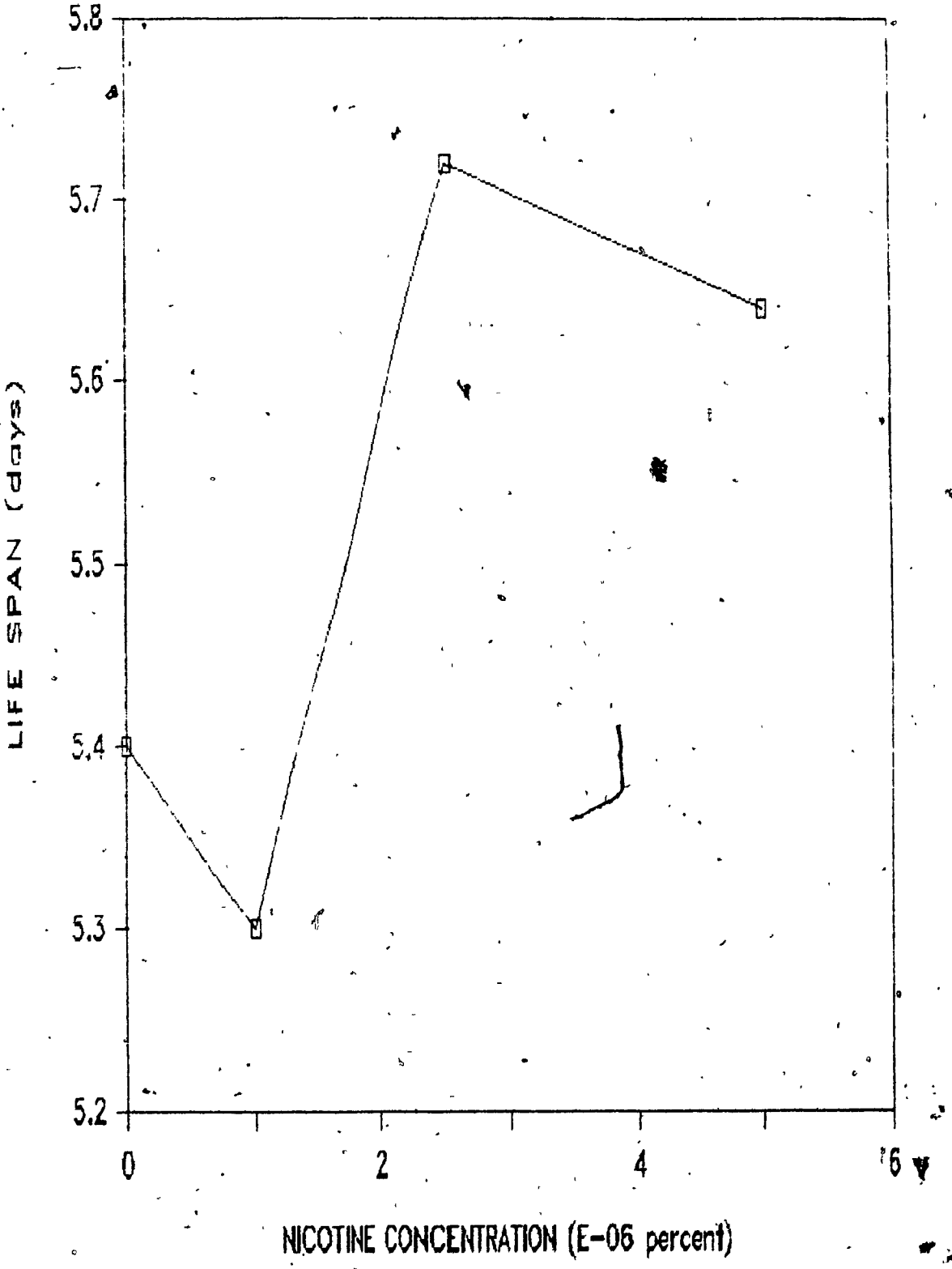

5.8
5.7
5.6
5.5
5.4
5.3
5.2
LIFE SPAN (days)
0
2
4
6
NICOTINE CONCENTRATION (E-06 percent)

Table 2

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT NICOTINE CONCENTRATIONS

Concentr. (%)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Ave. Offspring Number/ Rotifer
0	2.47 ± 0.06	2.47 ± 0.26	0.46 ± 0.08	7.71 ±0.75
0.000001	2.38 ± 0.09	2.24 ± 0.26	0.68 ± 0.11	6.96 ±0.69
0.0000025	2.34 ± 0.07	2.83 ± 0.25	0.55 ± 0.14	8.30 ±0.53
0.000005	2.47 ± 0.05	2.66 ± 0.23	0.51 ± 0.13	8.00 ±0.52

-No significant difference between any group..

Procaine

Longevity

The longevity data is shown in Table 3; the values indicate that rotifer lifespan is not influenced by any of the procaine concentrations ranging from 0.00001 to 0.0005 %. No significant differences occured between the control rotifers and the procaine-treated rotifers as shown by one-way analysis of variance ($(F\ 6, 161) = 2.635$, $p>0.05$). The graphs in Figures 3, 4 and 5 exhibit the relationship between the lifespan data and procaine concentration. Survival curves of control and procaine-treated groups are shown in Figures 3 and 4. The curves are all very close together indicating that rotifer survival was not altered by any procaine concentration. In Figure 5, the graph shows that lifespan does not change significantly from the control as the procaine concentration was increased; the points fall within a small lifespan range of 5 - 5.5 days.

The reproductive profile data is depicted in Table 4. The periods and offspring number of the reproductive profile were not significantly different from the control

Table 3

THE EFFECT OF PROCAINE ON THE LIFE-SPAN OF A. BRIGHTWELLI (N = 24)

Concentration of Procaine solution (percent)	Maximum Longevity (Days)	Mean Lifespan ± S.E.M. (Days)
0	6.75	5.38 ± 0.15
0.00001	6.75	5.43 ± 0.14
0.000025	6.75	5.48 ± 0.16
0.00005	6.00	5.30 ± 0.13
0.0001	7.00	5.46 ± 0.22
0.00025	7.00	5.39 ± 0.24
0.0005	6.75	5.03 ± 0.16

-No significant difference between any group

FIGURE 3. Survivorship curves of
A. brightwelli exposed to the lower range of procaine
concentrations as compared to control. (n = 24)

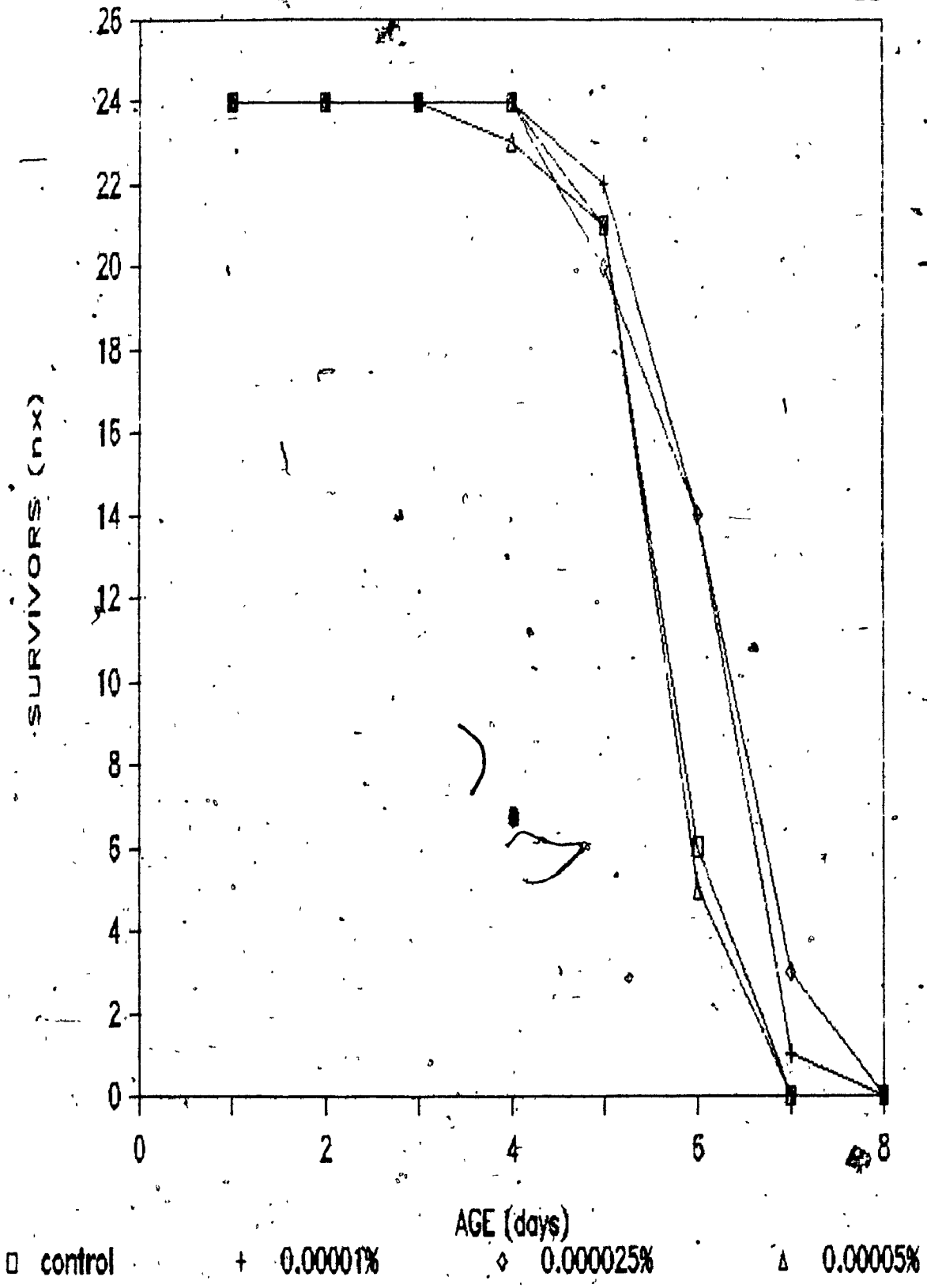

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
0.00001%
0.000025%
0.00005%

FIGURE 4. Survivorship curves of
A. brightwelli exposed to the higher range of procaine concentrations as compared to control. (n = 24)

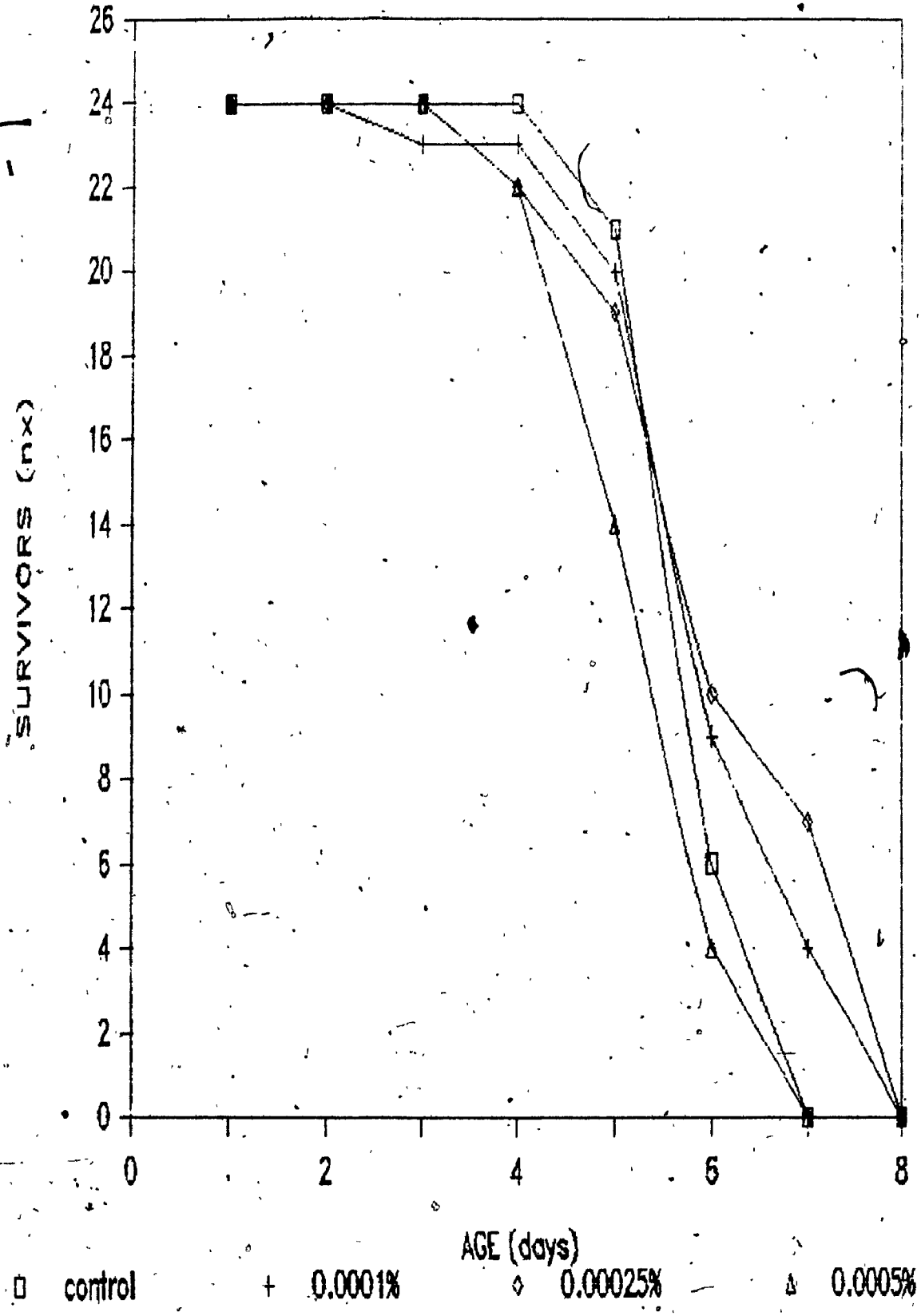

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
0.0001%
0.00025%
0.0005%

FIGURE 5. Life-span versus procaine concentration graph of A. brightwelli exposed to different procaine concentrations. (n = 24)

E-05 percent = 10^{-5} %

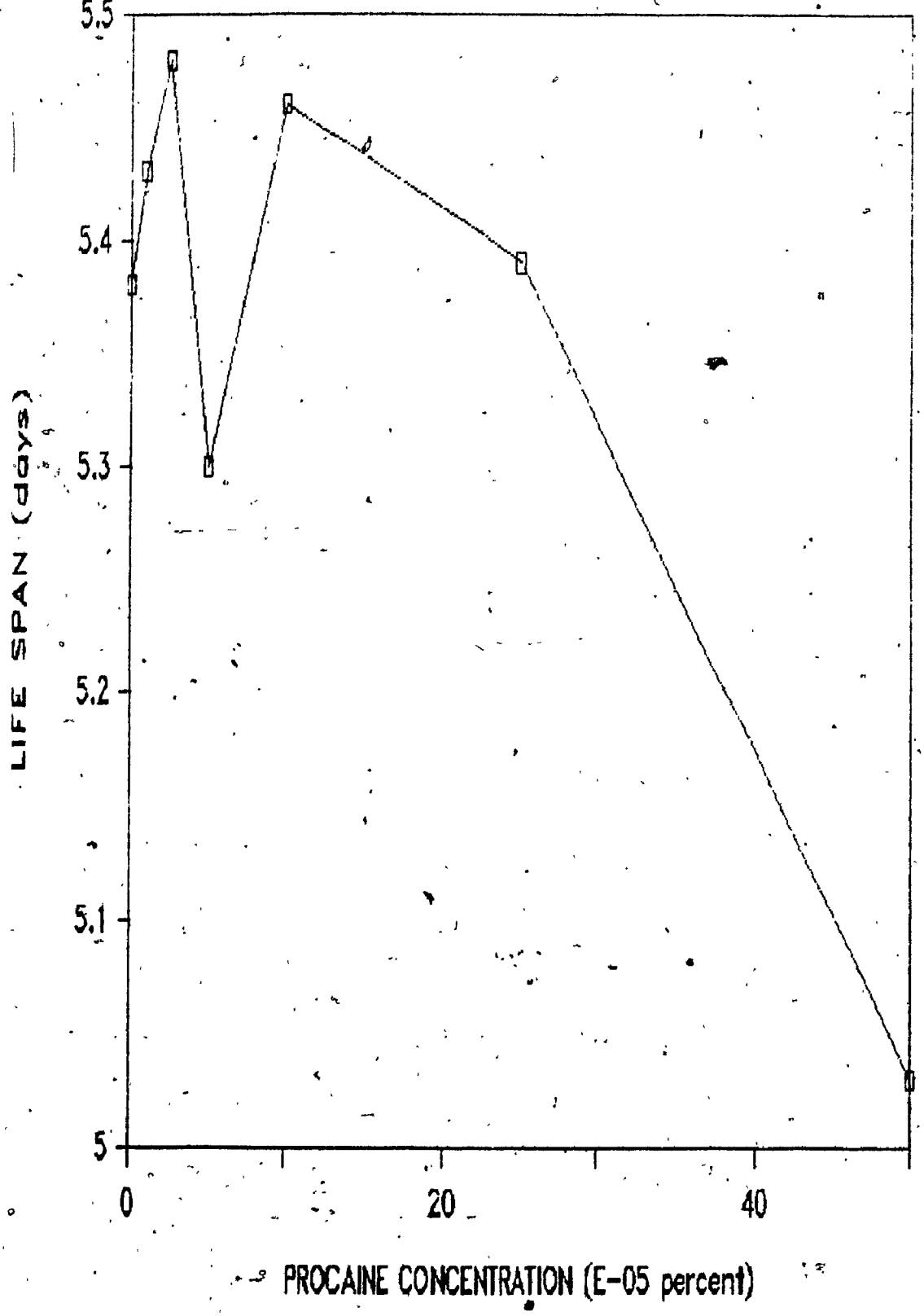

5.5
5.4
5.3
5.2
5.1
5
LIFE SPAN (days)
0
20
40
PROCAINE CONCENTRATION (E-05 percent)

Table 4

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT PROCAINE CONCENTRATIONS

Concentr. (%)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Ave. Offspring Number/ Rotifer
0	2.92 ± 0.07	2.09 ± 0.17	0.37 ± 0.07	6.63 ± 0.51
0.00001	2.63 ± 0.08	2.41 ± 0.14	0.39 ± 0.07	7.00 ± 0.48
0.000025	2.60 ± 0.06	2.47 ± 0.14	0.41 ± 0.07	7.79 ± 0.57
0.00005	2.63 ± 0.05	2.33 ± 0.18	0.34 ± 0.07	6.50 ± 0.50
0.0001	3.08 ± 0.13	2.14 ± 0.15	0.24 ± 0.04	5.71 ± 0.73
0.00025	3.09 ± 0.07	1.99 ± 0.24	0.31 ± 0.09	5.54 ± 0.54
0.0005	3.01 ± 0.08	1.67 ± 0.19	0.35 ± 0.06	5.25 ± 0.46

-No significant difference between any group

at any of the procaine concentrations, as determined by one-way analysis of variance and the post hoc Tukey test.

Atropine

Longevity

The influence of the atropine concentrations 0.0001, 0.00025 and 0.0005 % on rotifer lifespan as compared to the control are presented in Table 5. The data reveal that rotifers exposed to a 0.00025 % atropine solution have a significantly longer lifespan than any other group. A one-way analysis of variance showed that significant differences occured among the lifespan of the groups (F 3, 92) = 4.547, $p \leq 0.05$). The mean lifespan data is exhibited in Table 5. Further analysis by the post hoc Tukey test showed that rotifer lifespan was significantly increased by 0.00025 % atropine (6.10 $\pm$ 0.13 days) as compared with the control (5.70 $\pm$ 0.11 days).

Figures 6 and 7 elaborate graphically the different relationships in the lifespan data. As previously mentioned, only rotifers exposed to an atropine concentration of 0.00025 % had a significantly increased lifespan.

Table 5

THE EFFECT OF ATROPINE ON THE LIFE-SPAN OF A. BRIGHTWELLI
(N = 24)

Concentration of Atropine solution (percent)	Maximum Longevity (Days)	Mean Lifespan ± S.E.M. (Days)
0	6.75	5.70 ± 0.11
0.0001	7.00	5.80 ± 0.13
0.00025	7.00	6.10 ± 0.13*
0.0005	6.75	5.26 ± 0.23

*Statistically significantly different from control.
alpha = 0.05

FIGURE 6. Survivorship curves of *A. brightwelli* exposed to different atropine concentrations as compared to control. (n = 24)

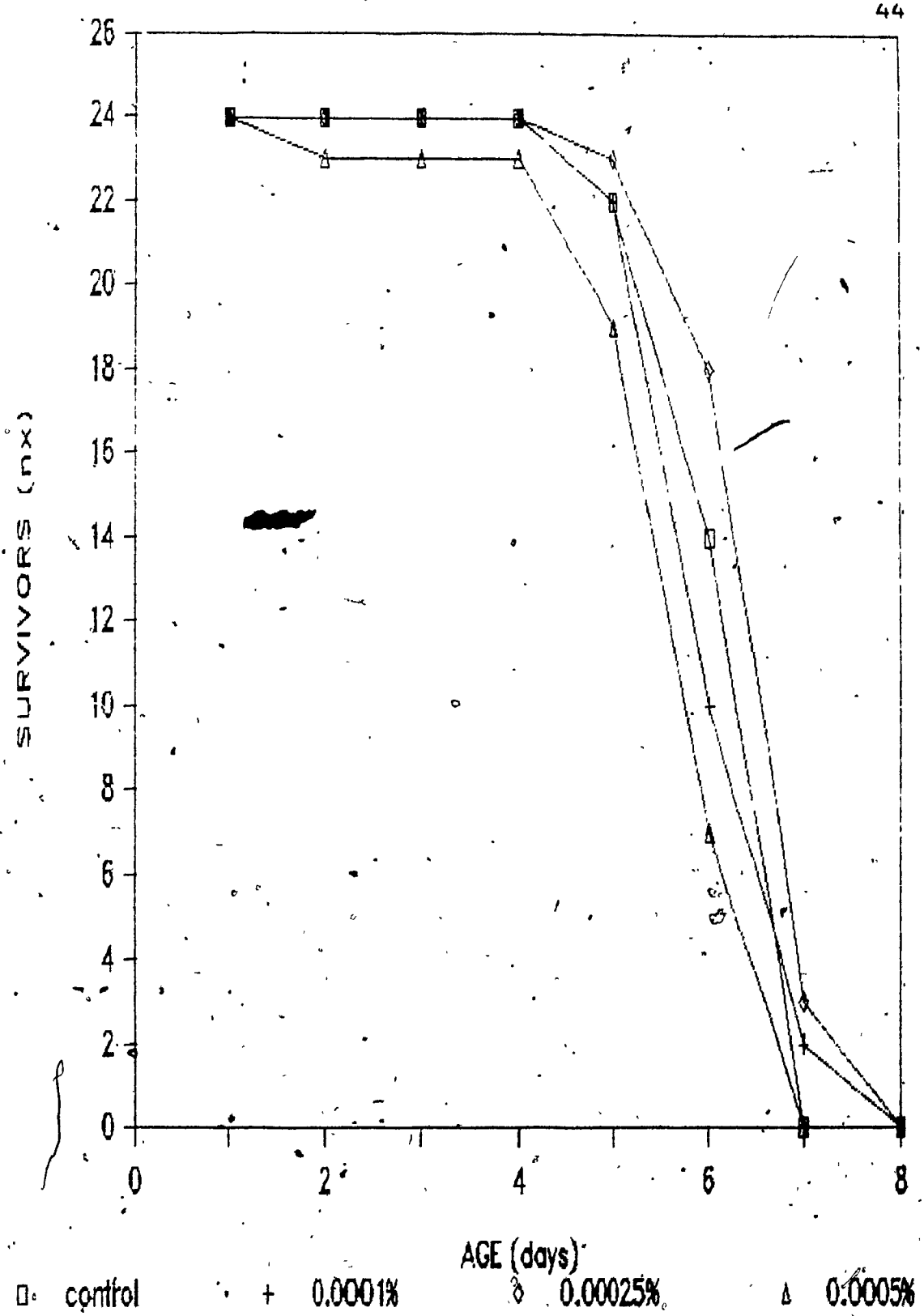

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
□ control
+ 0.0001%
◇ 0.00025%
△ 0.0005%

FIGURE 7. Life-span versus atropine concentration graph of A. brightwelli exposed to different atropine concentrations. (n = 24)

E-04 percent = 10^{-4} %

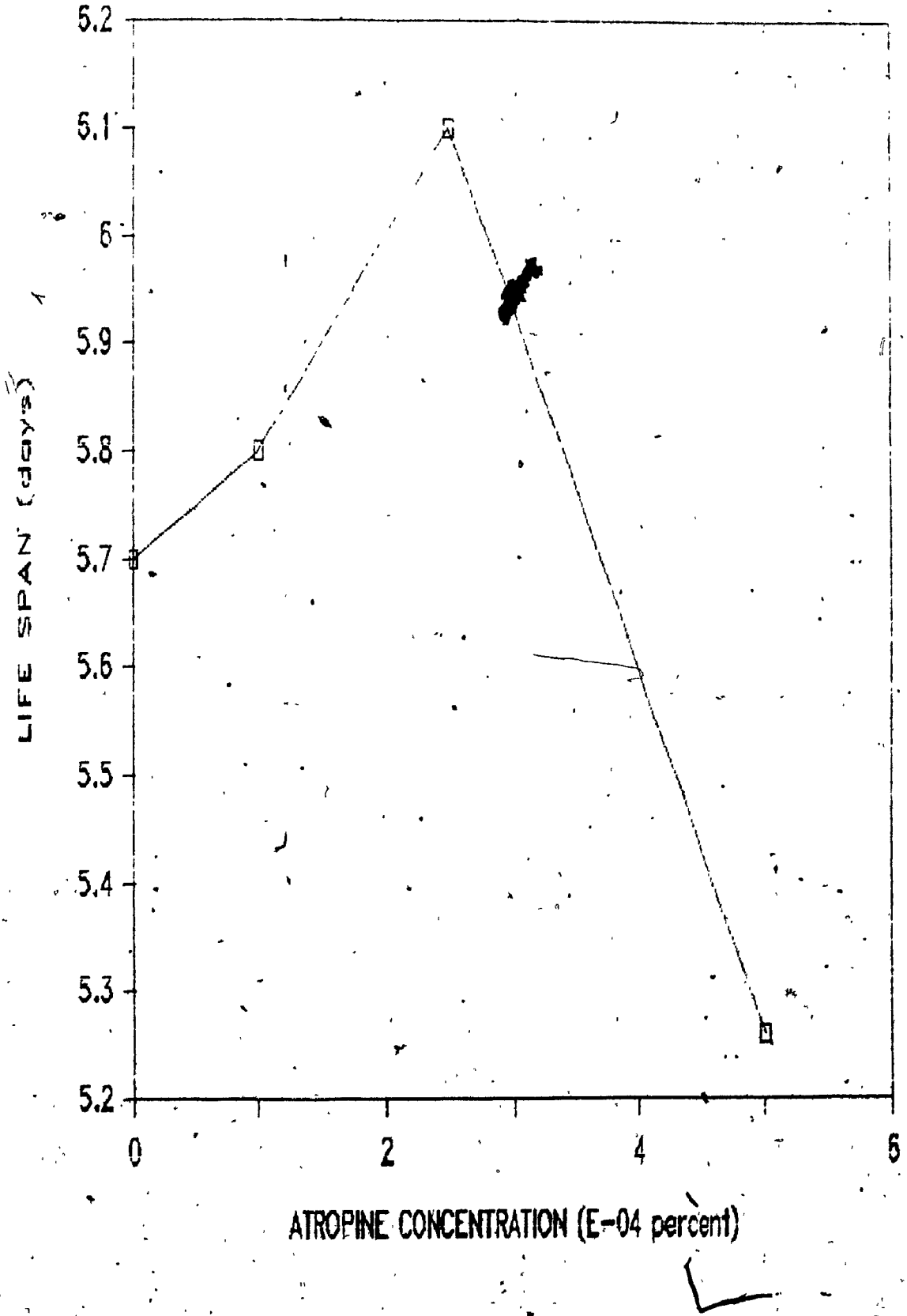

5.2
5.1
6
5.9
5.8
5.7
5.6
5.5
5.4
5.3
5.2
LIFE SPAN (days)
0
2
4
6
ATROPINE CONCENTRATION (E-04 percent)

Figure 6 shows the survival curves of the atropine groups and the control. The curve representing the rotifer group exposed to 0.00025 % of atropine lies furthest to the right, indicative of the longest lifespan. The lifespan seems to be lengthened starting at day 4. The graph of figure 7 reveals the effect of increasing atropine concentrations on lifespan. The graph indicates that rotifer lifespan steadily increases, reaching a peak at 0.00025 % and then decreases drastically. Thus the lifespan increase occurs over a very limited range of concentrations.

The reproductive profile is presented in Table 6. The table shows that a significant difference occurs only during the prereproductive period ($(F\ 3, 92) = 2.673$, $p>0.01$); this stage significantly increased at 0.00025 % atropine (3.16 $\pm$ 0.07 days) in comparison to the control (2.80 $\pm$ 0.14 days). Figure 8 shows each stage of the reproductive profile for all groups as a histogram to allow comparison between the groups as to the length of each stage of the life profile. The rotifers treated with 0.00025% atropine concentration clearly displays the longest lifespan. Figure 8 emphasizes that the increase in lifespan can be explained by an increase in the length of the prereproductive period. It can be noted that the

Table 6

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT ATROPINE CONCENTRATIONS

Concentr. (%)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Ave. Offspring Number/ Rotifer
0.	2.80 ± 0.14	2.57 ± 0.18	0.33 ± 0.05	7.67 ± 0.64
0.0001	2.91 ± 0.06	2.59 ± 0.19	0.30 ± 0.09	7.63 ± 0.52
0.00025	3.16 ± 0.07*	2.73 ± 0.16	0.21 ± 0.07	8.70 ± 0.52
0.0005	3.01 ± 0.08	2.20 ± 0.18	0.05 ± 0.05	6.17 ± 0.58

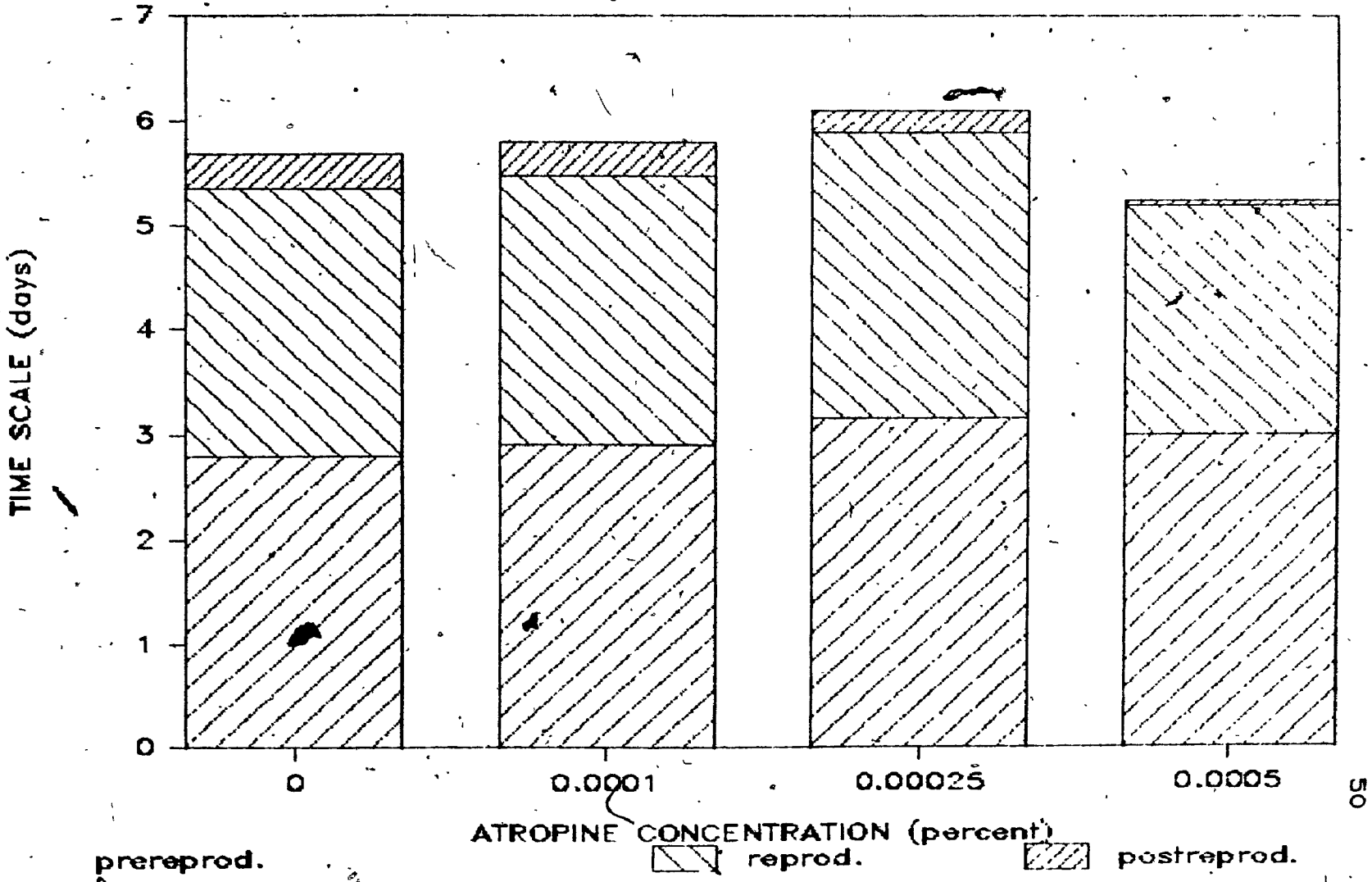
* Statistically significantly different from the control.
alpha = 0.05

FIGURE 8. Life profiles of A. brightwelli exposed to different atropine concentrations are expressed as histograms (stacked-bar graphs). (n = 24)

prereprod. = prereproductive period

reprod. = reproductive period

postreprod. = postreproductive period


7
6
5
4
3
2
1
0
TIME SCALE (days)
0
0.0001
0.00025
0.0005
ATROPINE CONCENTRATION (percent)
prereprod.
reprod.
postreprod.

length of the reproductive period remains quite constant regardless of treatment or lifespan.

Activity Level

Since the lifespan of the rotifer was increased at an atropine concentration of 0.00025%, further experiments were performed using this optimum atropine concentration to explain the lifespan increase.

In this section, the question of whether the lifespan increase was due to a change in the rotifer's activity level was examined. This was examined by doing movement counts. The data is displayed in Table 7. A one-way analysis of variance indicated that no significant differences in movement (number of squares traversed in one minute) on any day, between the control group and rotifers treated at the different atropine concentrations occured ($(F\ 3, 92) = 1.131$, $p>0.05$). The mean movement counts are shown in Table 7. The post hoc Tukey test showed that the movement counts were not significantly different from each other at any concentration on any day, yet overall activity decreased with age.

To further emphasize the lack of effect of atropine

Table 7

THE EFFECT OF ATROPINE ON THE MEAN MOVEMENT COUNT
(N = 24)

Concentr. Atropine (%)	Day 1	Day 2	Day 3
0	13.83 + 0.92	14.79 + 0.93	12.63 + 0.97
0.0001	15.67 + 0.69	14.59 + 0.80	15.42 + 0.85
0.00025	14.04 + 0.84	13.29 + 0.98	13.92 + 0.85
0.0005	15.04 + 0.77	16.46 + 0.75	14.00 + 0.71

-No statistically significant differences between any groups.

Concentr. Atropine (%)	Day 4	Day 5	Day 6
0	8.63 + 0.87	2.50 + 0.55	0.12 + 0.04
0.0001	11.63 + 1.02	2.52 + 0.41	0.13 + 0.04
0.00025	9.50 + 0.99	3.44 + 0.65	0.05 + 0.03
0.0005	10.50 + 0.78	1.83 + 0.48	0.03 + 0.02

-No statistically significant differences between any groups.

on activity, movement counts versus days was plotted in Figure 9. This graphic presentation shows that all curves fall within the same area. A pronounced decline in activity level is seen in all groups with increasing age.

Neutral Red

The neutral red was added to the Paramecium medium, where it was first taken up by the paramecia and these organisms were then ingested by the rotifer. This process stained the rotifer's gut and thus indicated how much food had been ingested.

The control rotifers and atropine-treated rotifers were exposed to neutral red stained paramecia to determine whether the atropine had produced dietary restriction.

As is indicated in Table 8, all rotifer groups revealed a bright red coloration of the gut, except for rotifers treated with 0.00025 % of atropine; these organisms had a pink coloration of the gut. This would indicate that rotifers at 0.00025% atropine ate less, thus undergoing dietary restriction.

Appendix II shows photographs of the different degrees of gut coloration between the rotifers treated with 0.00025% atropine and the other groups.

FIGURE 9. Movement curves [average number of squares transversed by the rotifer per minute versus the age of the rotifer in days] of Asplanchna brightwelli exposed to different concentrations of atropine. (n = 24)

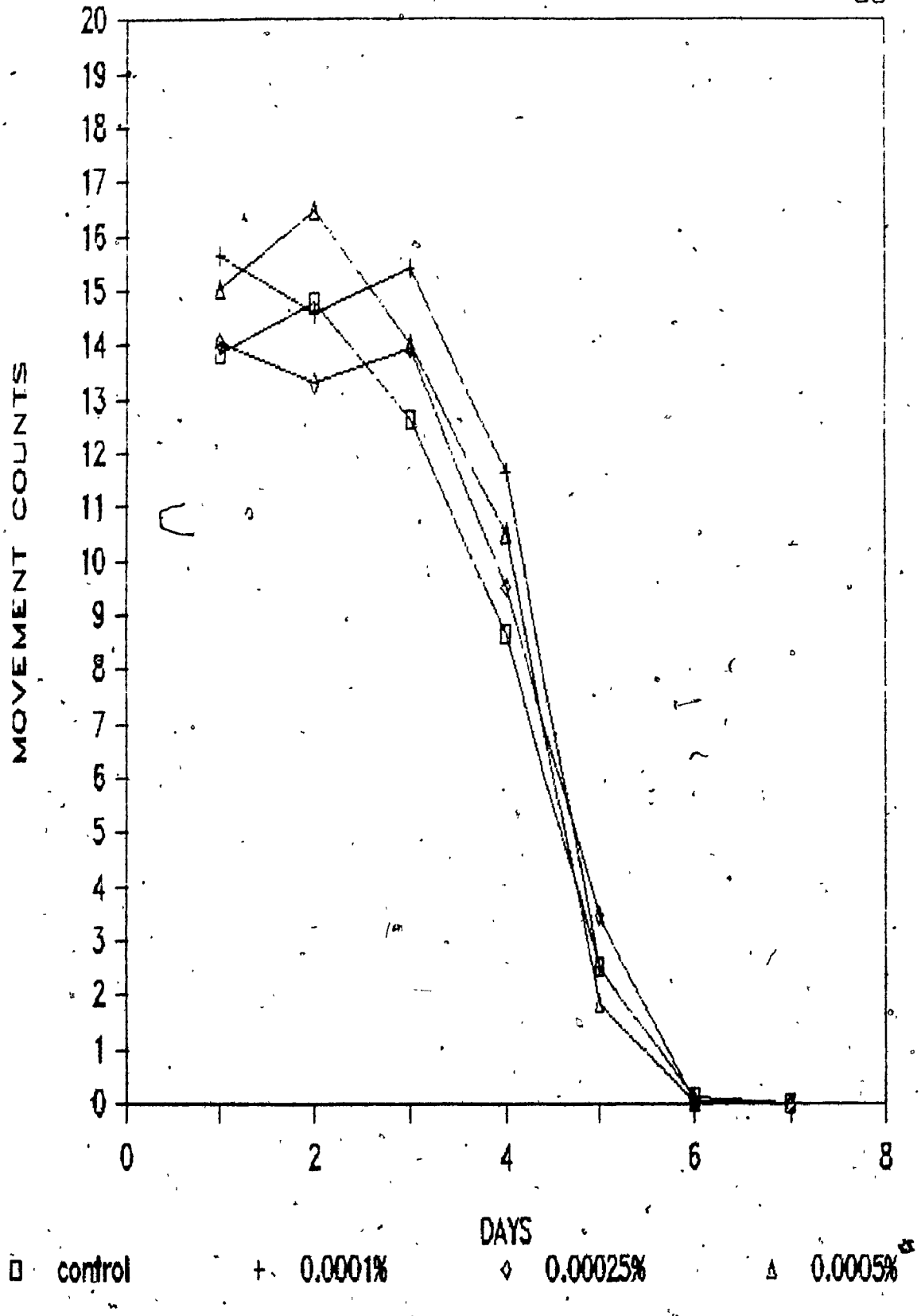

MOVEMENT COUNTS
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
2
4
6
8
DAYS
control
0.0001%
0.00025%
0.0005%

Table 8

THE EFFECT OF ATROPINE ON GUT COLORATION OF A. BRIGHTWELLI USING 0.75 ug/ml NEUTRAL RED

Atropine Concentration (percent)	Paramecium Density (paramecia/ml)	Gut Coloration after 2 days
0	300	bright red
0.0001	300	bright red
0.00025	300	pink

The Hormones

Determination of Optimum Hormone Concentration

The purpose of the experiments performed in this section was to determine whether or not various concentrations of specific hormones would modify the lifespan and fecundity of rotifers.

The hormones used were ~~thyroxine~~, B-estradiol, cortisone and hydrocortisone. These conpounds are not water sdluble, thus they were solubilized in 100 % ethanol for addition to the culture medium.

Thyroxine

Longevity

The longevity data for rotifers exposed to thyroxine concentrations ranging from 10 to 800 uM are displayed in Table 9. As can be seen from the table, the rotifer lifespan is not affected by any of the ~~thyroxine~~ concentrations. A one-way analysis of variance showed that no significant differences in lifespan between the control group and the rotifers exposed to the different

Table 9

THE EFFECT OF THYROXINE ON THE LIFESPAN OF A. BRIGHTWELLI
(N = 24)

Concentration of Thyroxine solution (micromolar)	Maximum Longevity (Days)	Mean Lifespan ± S.E.M. (Days)
0	7.50	5.71 ± 0.42
10	7.00	5.50 ± 0.44
50	6.00	5.00 ± 0.25
100	7.00	5.29 ± 0.32
200	5.50	5.04 ± 0.18
400	5.50	4.17 ± 0.65
800	5.25	4.08 ± 0.52

-No significant difference between any group.

thyroxine concentrations occured ((F 6, 161) = 1.990, $p \geq 0.05$). The mean lifespan values are shown in Table 9. Despite the absence of statistical differences, the mean lifespan of rotifers exposed to 400 and 800 uM of thyroxine is shorter than that of the control. This slight decrease in lifespan is most likely due to a toxicity effect on the rotifers; the thyroxine becomes toxic as the concentration is increased.

The relationship between the lifespan values can be further emphasized graphically as in Figures 10, 11 and 12. Figures 10 and 11 present the survival curves for control and thyroxine-treated rotifers. All the curves of Figure 10 which shows the effect of lower concentrations of thyroxine, lie within the same area except the 200 uM curve which lies further to the left on day 5 and 6. In Figure 11 which shows the effect of higher concentrations of thyroxine, the control curve lies further to the right than the survival curve for rotifers treated with 400 and 800 uM of thyroxine; fewer rotifers surviving at these two highest thyroxine concentrations. In Figure 12, lifespan is plotted versus thyroxine concentration. This graph shows that there is a small decrease in lifespan for rotifers treated with 400 and 800 uM thyroxine. This decrease in lifespan is evidently due to a toxic effect of

FIGURE 10. Survivorship curves of
A. brightwelli exposed to the lower range of thyroxine concentrations as compared to control. (n = 24)

control = 0 thyroxine concentration

10 uM = 10 micromolar thyroxine concentration

50 uM = 50 micromolar thyroxine concentration

100 uM = 100 micromolar thyroxine concentration

200 uM = 200 micromolar thyroxine concentration

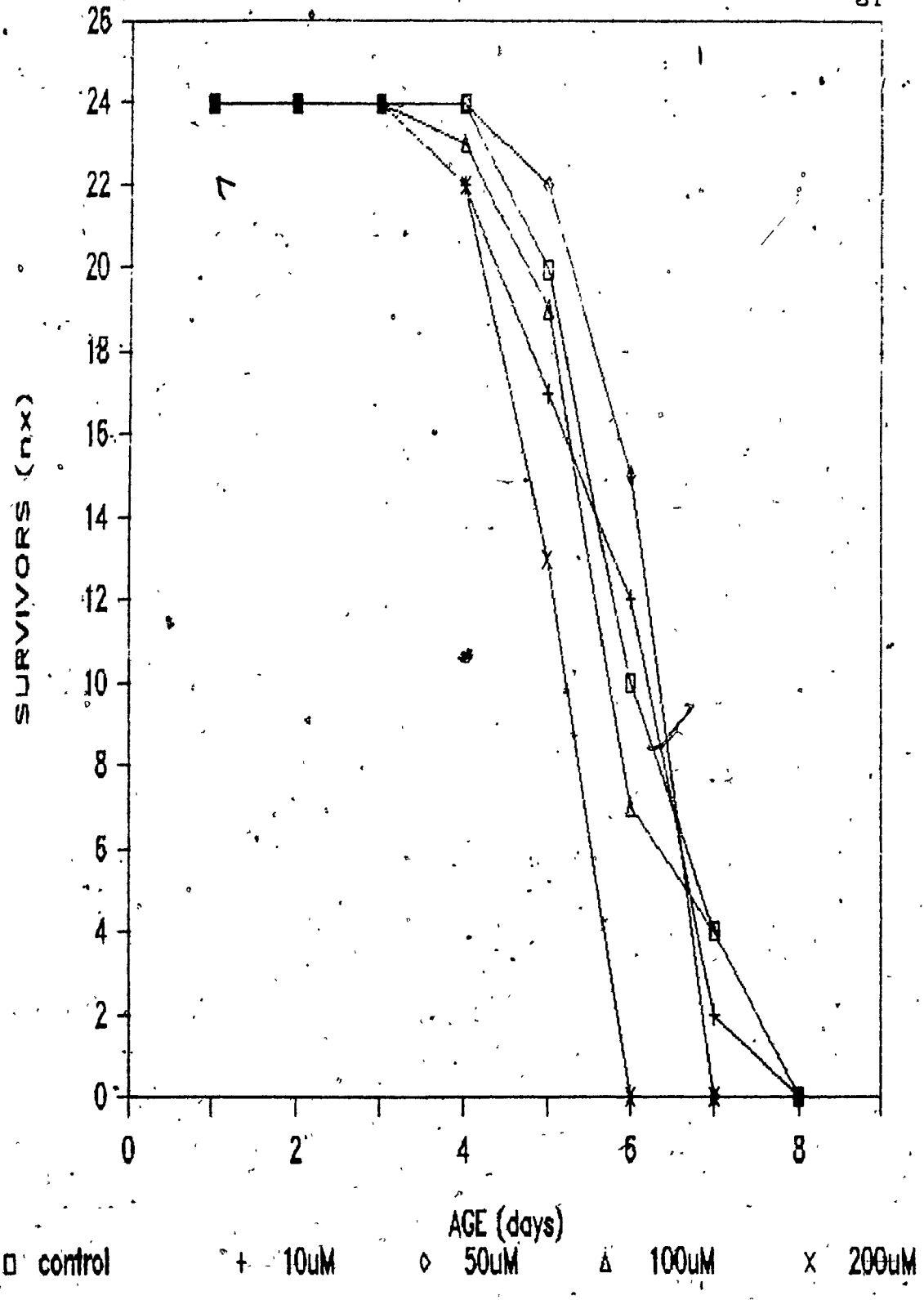

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
□ control
+ 10uM
◊ 50uM
Δ 100uM
X 200uM

FIGURE 11. Survivorship curves of A. brightwelli exposed to the higher range of thyroxine concentrations as compared to control. (n = 24)

control = 0 thyroxine concentration

400 uM = 400 micromolar thyroxine concentration

800 uM = 800 micromolar thyroxine concentration

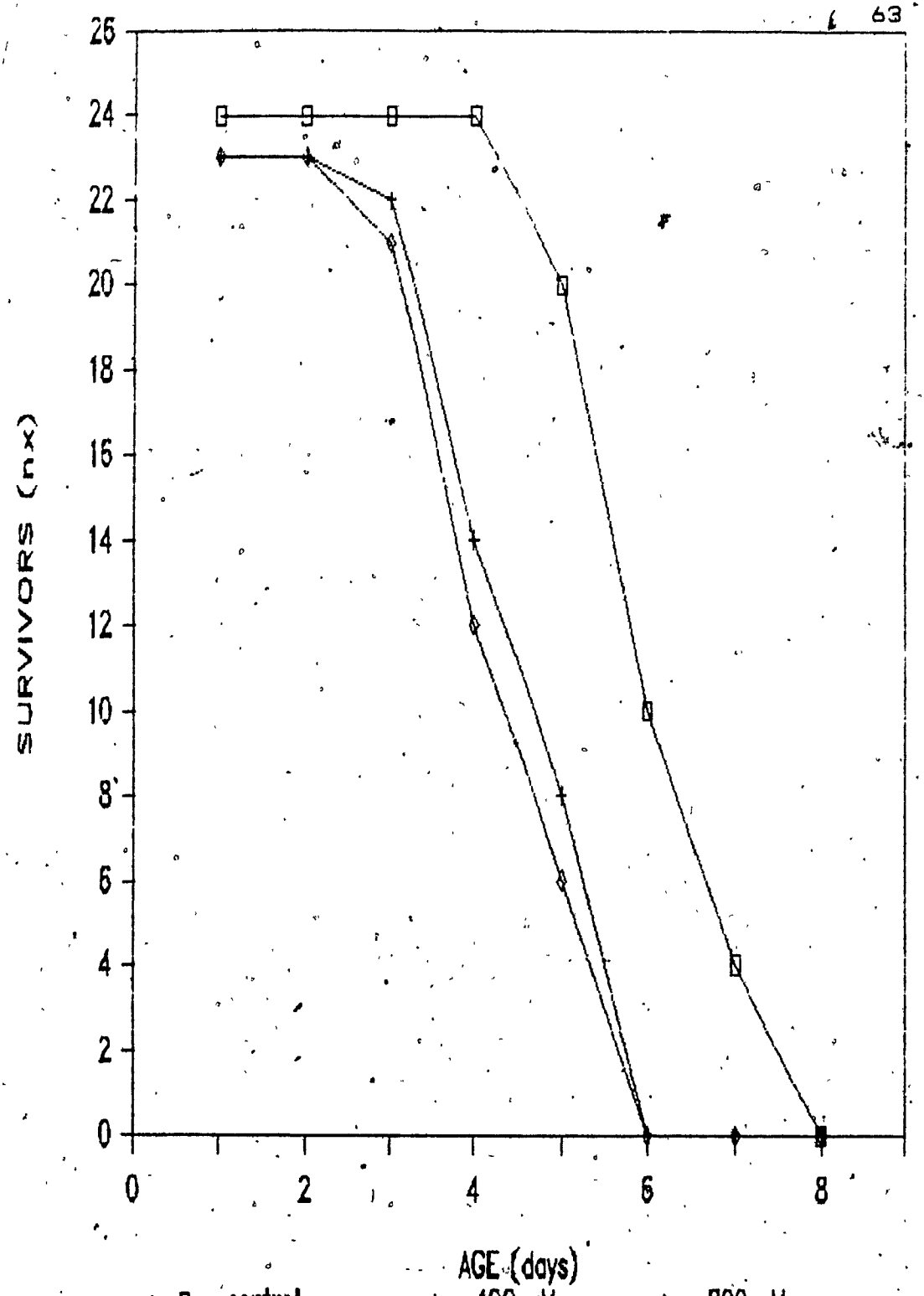
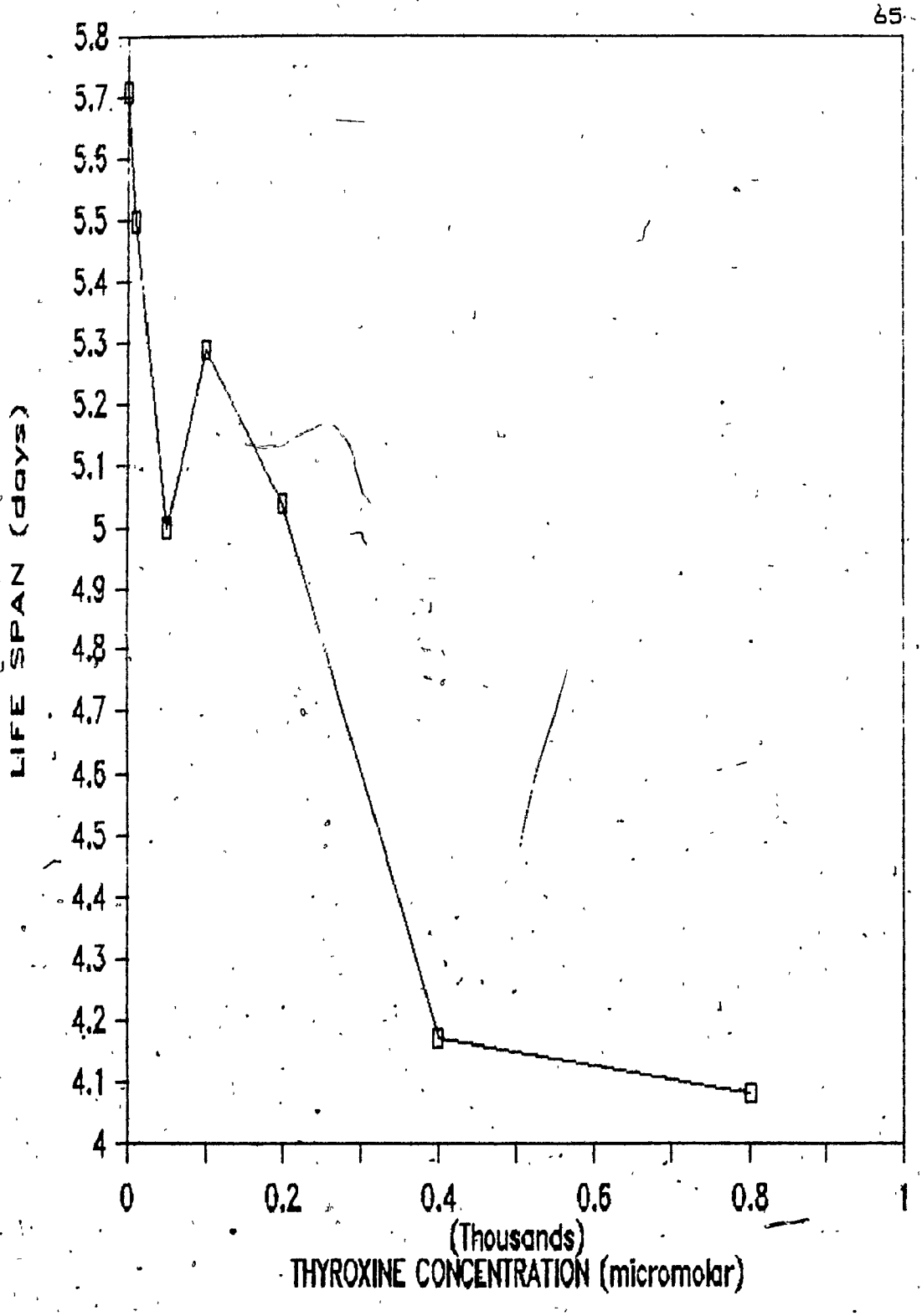

SURVIVORS (nx)
25
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
400 uM
800 uM

FIGURE 12. Average life-span versus thyroxine concentration graph of A. brightwelli exposed to different thyroxine concentrations. (n = 24)


LIFE SPAN (days)
5.8
5.7
5.6
5.5
5.4
5.3
5.2
5.1
5
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4
0
0.2
0.4
0.6
0.8
1
(Thousands)
THYROXINE CONCENTRATION (micromolar)

increasing increasing levels of thyroxine on the rotifers, but it is not statistically significant.

The reproductive profile of the rotifer, incorporating prereproductive, reproductive and postreproductive periods of the control and the rotifers treated with different thyroxine concentrations, is displayed in Table 10. This table shows that there are significant differences between the groups in the length of the reproductive period ($(F\ 6, 161) = 12.358$, $p \leq 0.01$); at a thyroxine concentration of 800 uM, this period is greatly reduced (0.38 $\pm$ 0.21 days) when compared to the control (1.92 $\pm$ 0.51 days). The offspring number ($(F\ 6, 161) = 6.673$, $p \leq 0.05$) of rotifers treated at 400 uM (2.50 $\pm$ 0.56) and 800 uM (1.33 $\pm$ 0.59) are significantly lower in comparison to the control (4.83 $\pm$ 0.42).

B-estradiol

Longevity

The average lifespan of rotifers exposed to various concentrations of B-estradiol is shown in Table 11; the values indicate that there is a steady decrease in rotifer lifespan as the B-estradiol concentrations are increased. Rotifer lifespan was shortest at B-estradiol

Table 10

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT CONCENTRATIONS OF THYROXINE

Concentr. (uM)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Ave. Offspring Number/ Rotifer
0	2.88 ± 0.13	1.92 ± 0.51	0.91 ± 0.43	4.83 ± 0.42
10	2.54 ± 0.10	2.04 ± 0.38	0.92 ± 0.21	5.50 ± 0.59
50	2.33 ± 0.11	2.12 ± 0.27	0.55 ± 0.29	5.17 ± 0.48
100	2.63 ± 0.14	2.37 ± 0.25	0.29 ± 0.24	5.50 ± 0.56
200	2.46 ± 0.11	1.71 ± 0.17	0.87 ± 0.30	4.17 ± 0.48
400	2.17 ± 0.23	0.83 ± 0.30*	1.17 ± 0.30*	2.50 ± 0.56*
800	2.92 ± 0.41	0.38 ± 0.21*	0.78 ± 0.39*	1.33 ± 0.49*

* Statistically significantly different from the control.
alpha = 0.01

Table 11

THE EFFECT OF B-ESTRADIOL ON THE LIFE-SPAN OF A. BRIGHTWELLI
(N = 24)

Concentration of B-estradiol solution (micromolar)	Maximum Longevity (Days)	Mean Lifespan ± S.E.M. (Days)
0	5.50	5.08 ± 0.14
10	5.00	4.83 ± 0.11
50	5.25	4.75 ± 0.35
100	4.75	4.13 ± 0.30
200	3.75	2.79 ± 0.35*
400	3.00	2.33 ± 0.42*
800	4.50	2.58 ± 0.55*

*Statistically significantly different from the control.
alpha = 0.01

concentrations of 200, 400 and 800 uM. A one-way analysis of variance showed that significant differences occured amoung the lifespan of the groups ($F_{6, 161} = 11.168$, $p \leq 0.01$). The mean lifespan data is exhibited in in Table 11. The post hoc Tukey test indicates that the rotifer lifespan was significantly decreased at 200 uM B-estradiol (2.79 $\pm$ 0.35 days), 400 uM (2.33 $\pm$ 0.42 days) and 800 uM (2.58 $\pm$ 0.55 days) compared with the control (5.08 $\pm$ 0.14 days).

The Figures 13, 14 and 15 elaborate graphically the different relationships in the lifespan data. Figure 13 shows the survival curves of rotifers exposed to lower levels of B-estradiol as compared to the control. The curves representing the rotifer groups exposed to 100 and 200 uM of B-estradiol lie very far to the left as compared to the control. Figure 14 shows the survival curves of rotifers exposed to 400 and 800 uM B-estradiol. The survival curves of these two groups lie furthest to the left, indicative of the shortest lifespan. It is important to realize that the entire lifespan is affected at the higher concentrations of B-estradiol. The graph of Figure 15 shows the effect of increasing B-estradiol concentrations on rotifer lifespan. This graph indicates that rotifer lifespan is steadily decreasing as B-

FIGURE 13. Survivorship curves of
A. brightwelli exposed to the lower range of B-estradiol concentrations as compared to control. (n = 24)

control = 0 B-estradiol concentration

10 uM = 10 micromolar B-estradiol concentration

50 uM = 50 micromolar B-estradiol concentration

100 uM = 100 micromolar B-estradiol concentration

200 uM = 200 micromolar B-estradiol concentration

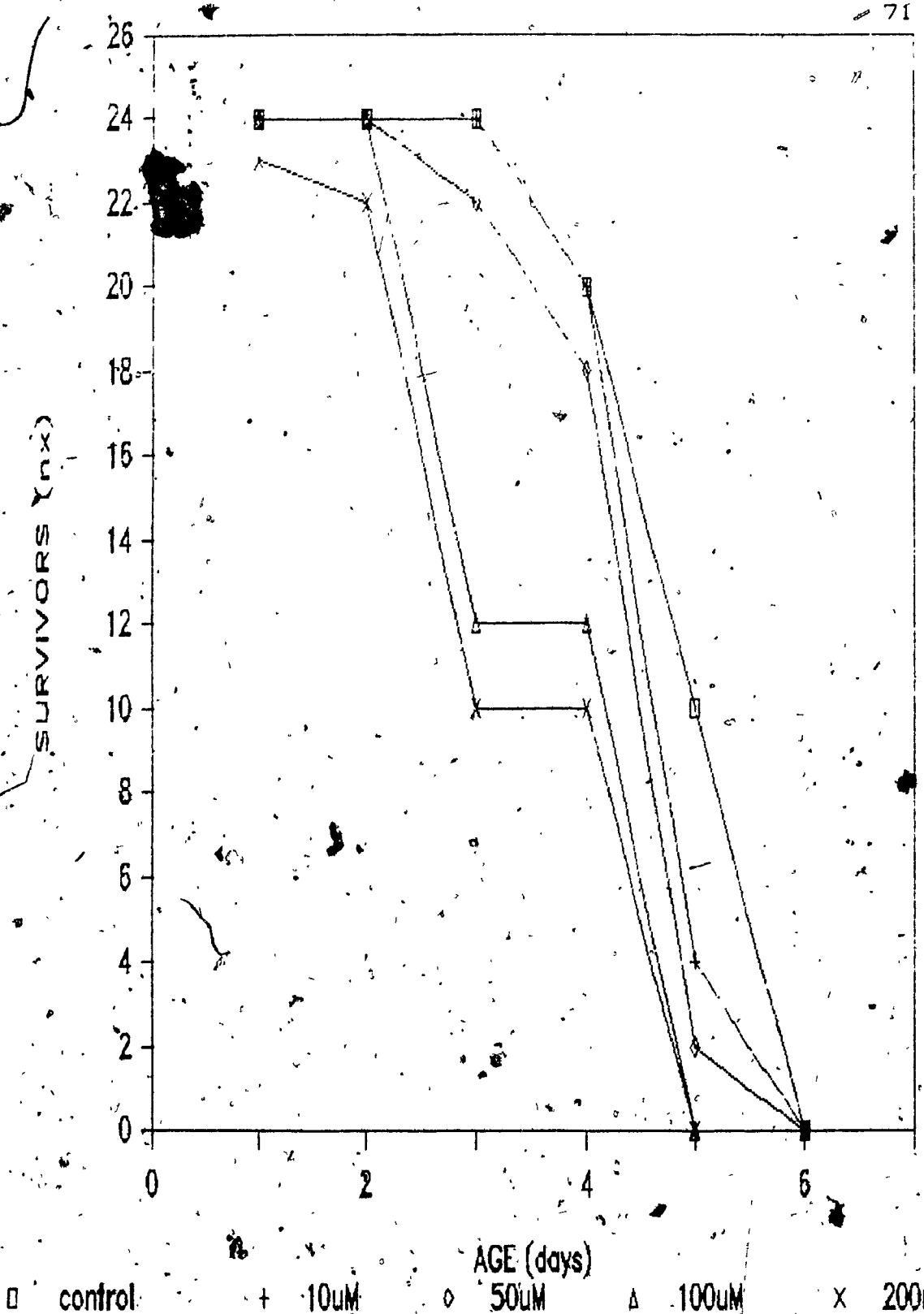

SURVIVORS (n x)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
AGE (days)
control
10uM
50uM
100uM
200uM

FIGURE 14. Survivorship courves of A. brightwelli exposed to the higher range of B-estradiol concentrations as compared to control. (n = 24)

control = 0 B-estradiol concentration

400 uM = 400 micromolar B-estradiol concentration

800 uM = 800 micromolar B-estradiol concentration

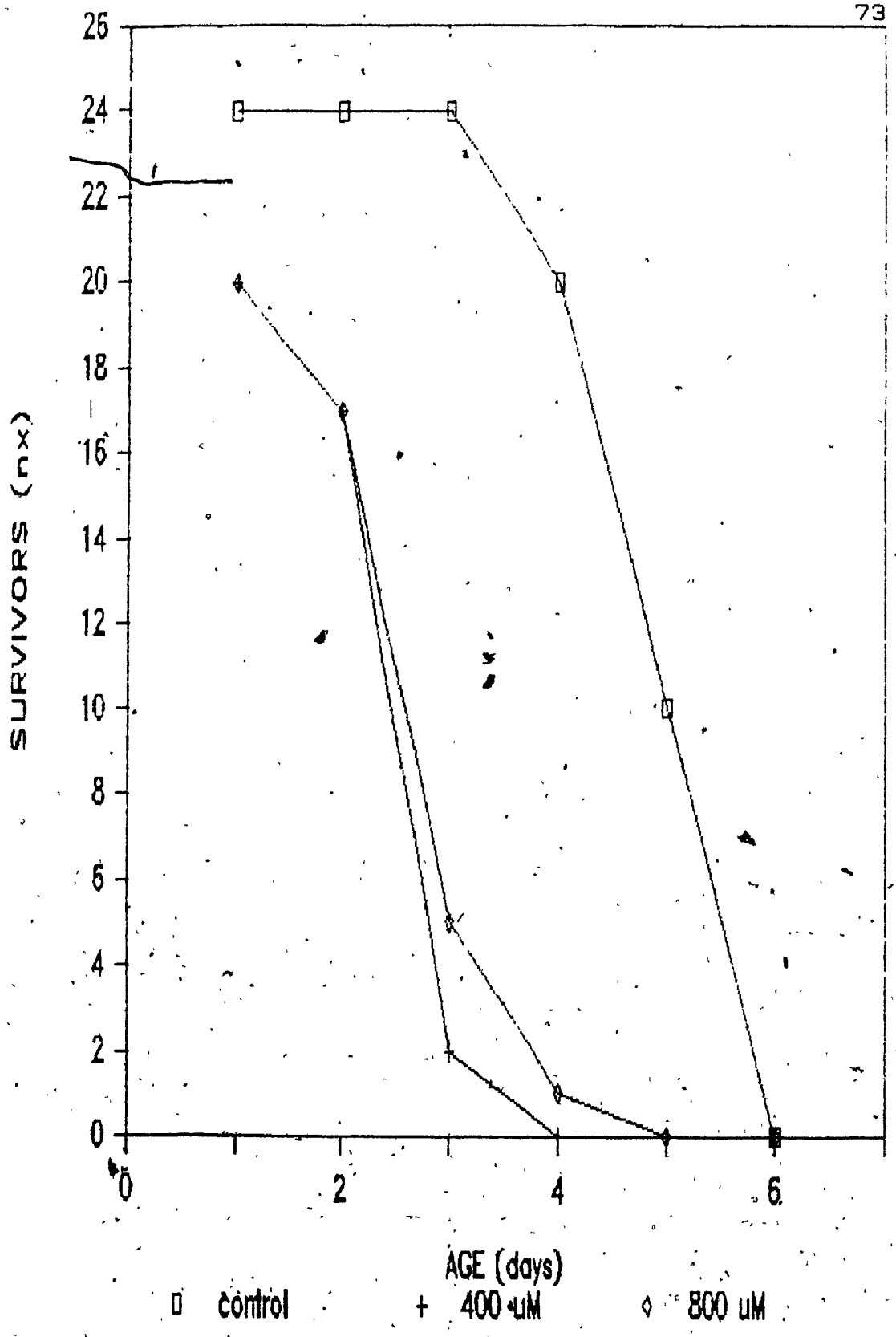
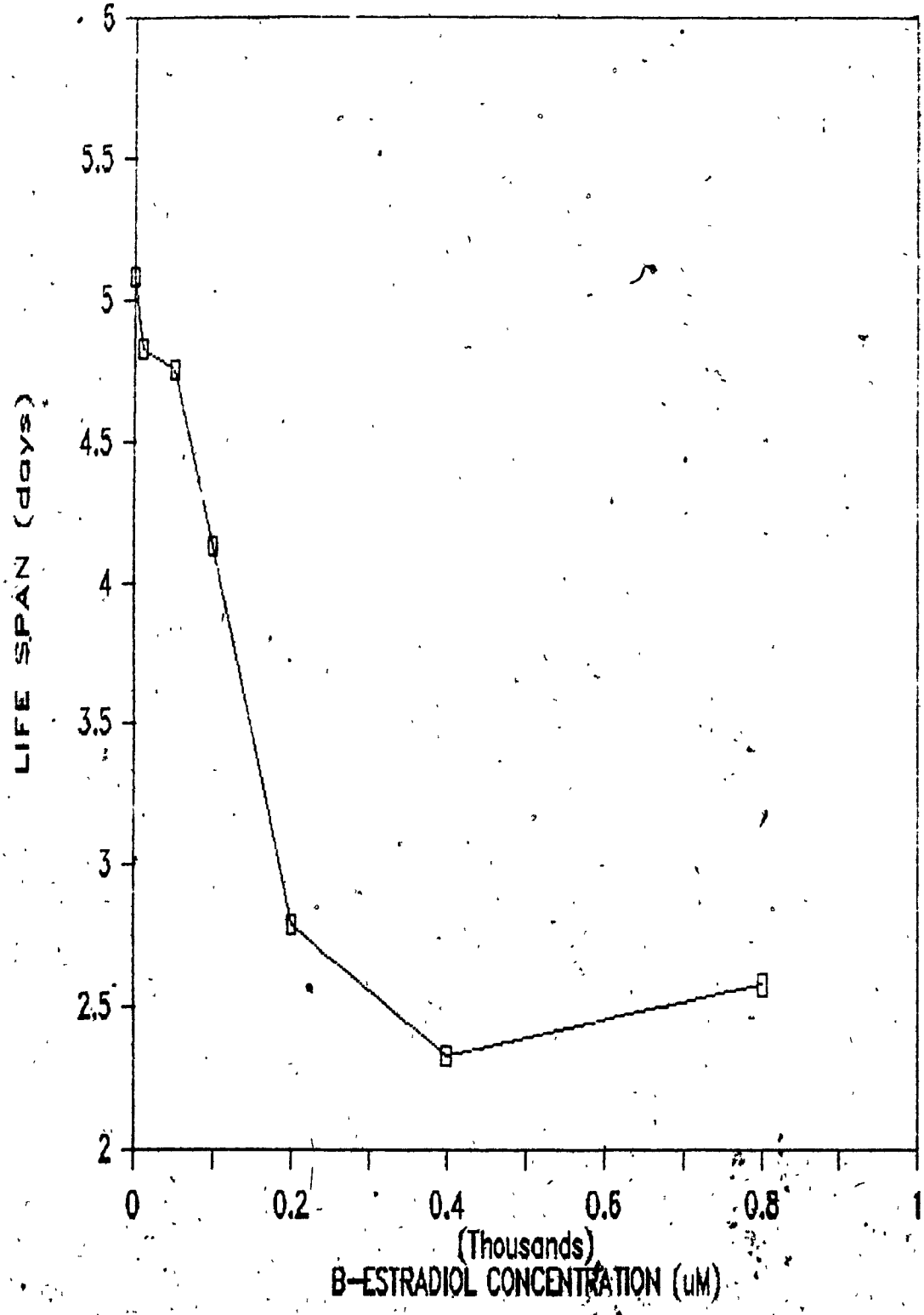

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
AGE (days)
control
400 uM
800 uM

FIGURE 15. Average life-span versus B-estradiol concentration graph of A. brightwelli exposed to different B-estradiol concentrations. (n = 24)


LIFE SPAN (days)
6
5.5
5
4.5
4
3.5
3
2.5
2
0
0.2
0.4
0.6
0.8
1
(Thousands)
B-ESTRADIOL CONCENTRATION (uM)

estradiol concentration increases.

The reproductive profile of rotifers exposed to different concentrations of B-estradiol is presented in Table 12. The table shows that there are significant differences in the length of the reproductive period ((F 6, 161) = 20.361, $p \leq 0.01$); this stage is significantly decreased at 200 uM B-estradiol (0.12 ± 0.13 days), 400 uM (0.12 ± 0.13 days) and 800 uM (0 days) in comparison to the control (2.21 ± 0.14 days). The number of offspring ((F 6, 35) = 14.713, $p \leq 0.01$) produced by the rotifers is also influenced by B-estradiol. The offspring number decreases significantly at 100 uM (2.50 ± 0.67), 200 uM (0.33 ± 0.33), 400 uM (0.17 ± 0.17) and 800 uM (0) when compared to the control (5.83 ± 0.60). The data in Table 12 also show that the postreproductive period is entirely eliminated at the three highest B-estradiol concentrations.

Cortisone

Longevity

The influence of the cortisone concentrations, ranging from 10 to 800 uM, on rotifer lifespan as compared to the control are presented in Table 13. From the table

Table 12

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT B-ESTRADIOL CONCENTRATIONS

Concentr. (uM)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Ave. Offspring Number/ Rotifer
0	2.71 ± 0.08	2.21 ± 0.14	0.16 + 0.08	5.83 ± 0.60
10	2.75 ± 0.00	2.21 ± 0.10	0.13 + 0.04	5.50 ± 1.09
50	2.67 ± 0.08	1.92 ± 0.42	0.16 + 0.07	4.83 ± 1.08
100	2.75 ± 0.22	1.13 ± 0.35	0.25 + 0.07	2.50 ± 0.67
200	2.67 ± 0.33	0.12 ± 0.13*	0	0.33 ± 0.33*
400	2.21 ± 0.40	0.12 ± 0.13*	0	0.17 ± 0.17*
800	2.58 ± 0.55	0	0	0

* Statistically significantly different from the control. alpha = 0.01

Table 13

THE EFFECT OF CORTISONE ON THE LIFE-SPAN OF A. BRIGHTWELLI
(N = 24)

Concentration of Cortisone solution (micromolar)	Maximum Longevity (Days)	Mean Lifespan ± S.E.M. (Days)
0	7.0	5.38 ± 0.32
10	6.5	5.42 ± 0.27
50	7.0	5.50 ± 0.50
100	7.5	6.50 ± 0.41*
200	7.0	6.42 ± 0.29*
400	6.0	5.21 ± 0.25
800	6.0	5.04 ± 0.45

*Statistically significantly different from the control.
alpha = 0.01

it is apparent that the lifespan of rotifers exposed to 100 uM and 200 uM cortisone solution is significantly prolonged. A one-way analysis of variance showed that significant differences in the length of the lifespan occured amoung the groups ($(F\ 6,161) = 7.153$, $p \leq 0.01$). The post hoc Tukey test showed that the lifespan of the rotifers treated with 100 uM cortisone (6.50 $\pm$ 0.41 days) and 200 uM cortisone (6.42 $\pm$ 0.29 days) was significantly longer as compared to the mean lifespan of the controls (5.38 $\pm$ 0.32 days).

The graphs in Figures 16, 17 and 18 exhibit the relationship between the lifespan data of the various groups. Survival curves of control and cortisone-treated groups are shown in Figures 16 and 17. Figure 16 shows the survival curves of rotifers treated with the four lowest levels of cortisone used. Figure 16 reveals that the curves at 100 and 200 uM are shifted farthest to the right denoting the longest lifespan. Figure 17 shows the survival curves of the rotifers treated with the two highest levels of cortisone used. The curves of Figure 17 are all very close together, showing that cortisone at these higher concentrations had very little effect on survivorship. In Figure 18, the graph indicates that rotifer lifespan first increases, reaching a peak at 100

FIGURE 16. Survivorship curves of A. brightwelli exposed to the lower range of cortisone concentrations as compared to control. (n = 24)

control = 0 cortisone concentration

10 uM = 10 micromolar cortisone concentration

50 uM = 50 micromolar cortisone concentration

100 uM = 100 micromolar cortisone concentration

200 uM = 200 micromolar cortisone concentration

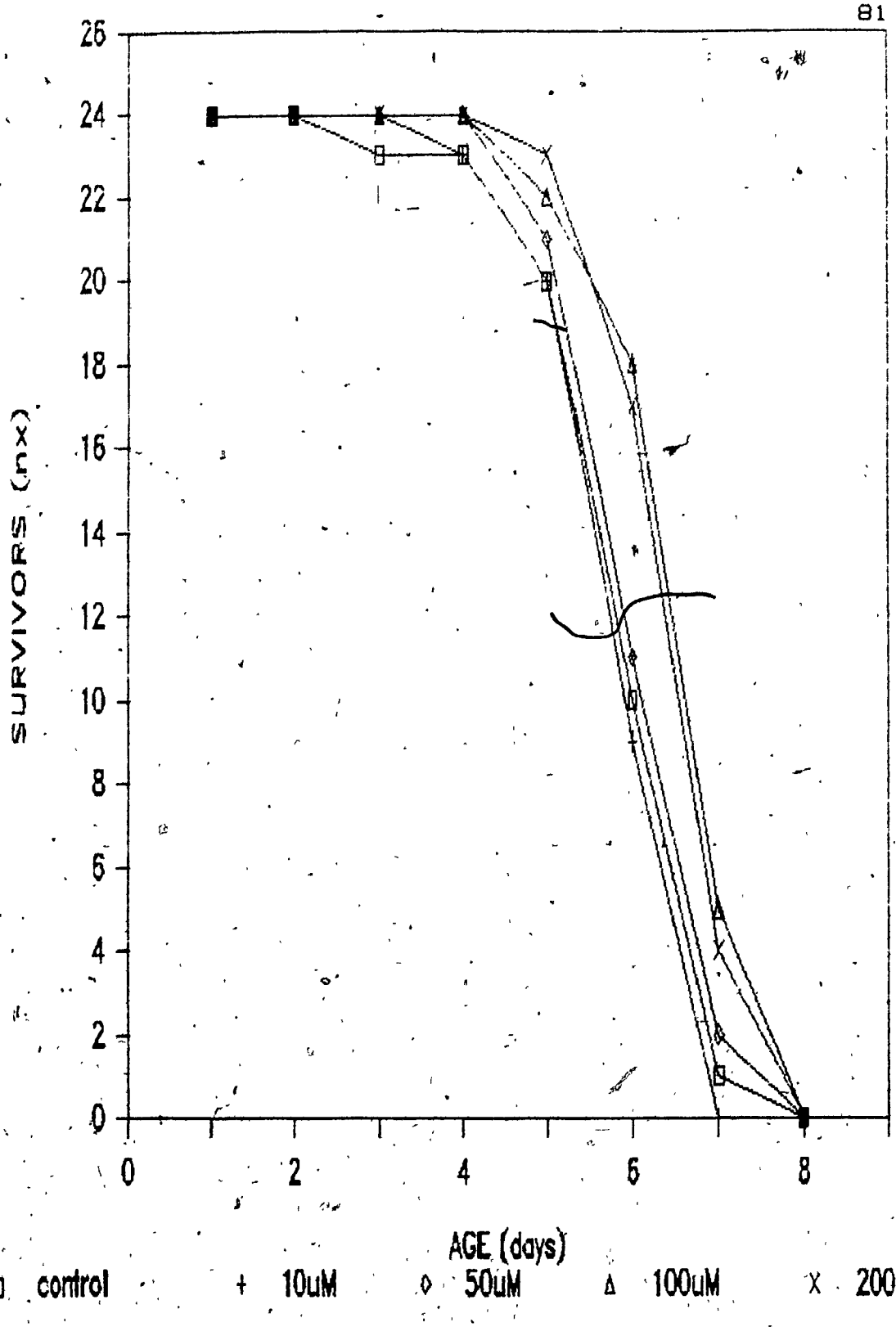

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
10uM
50uM
100uM
200uM

FIGURE 17. Survivorship curves of A. brightwelli exposed the higher range of cortisone concentrations as compared to control. (n = 24)

control = 0 cortisone concentration

400 uM = 400 micromolar cortisone concentration

800 uM = 800 micromolar cortisone concentration

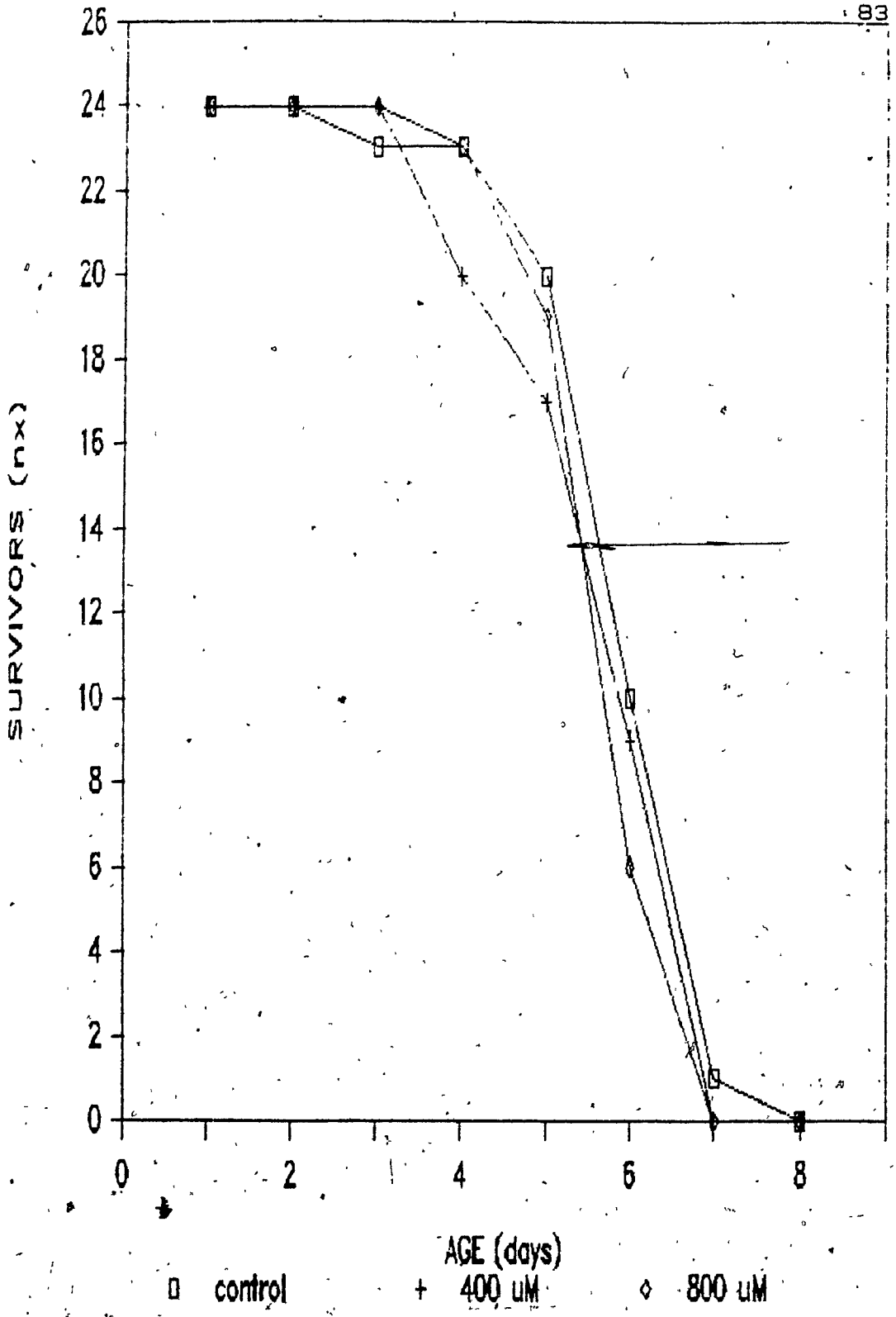
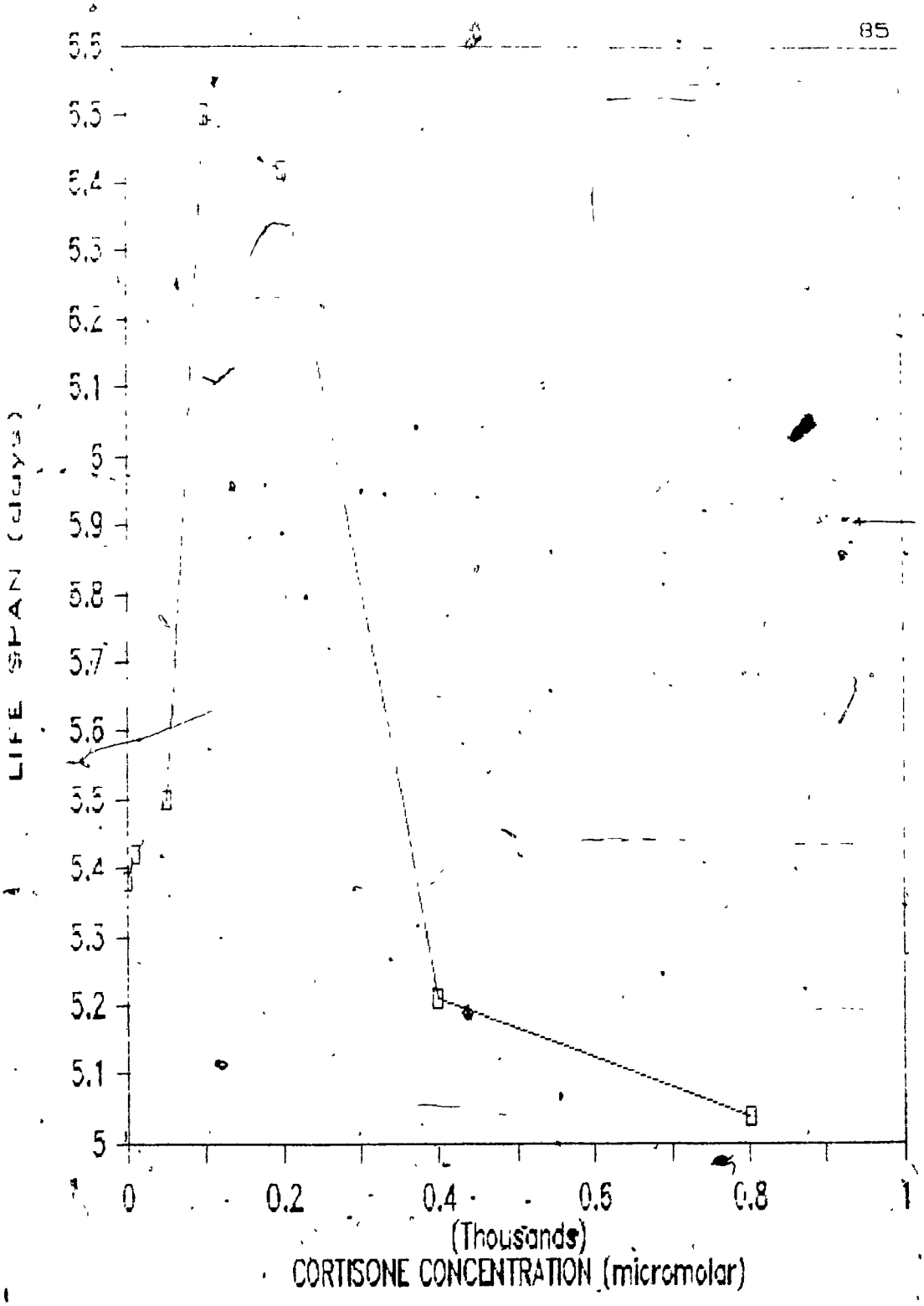

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
400 uM
800 uM

FIGURE 18. Average life-span versus cortisone concentration graph of A. brightwelli exposed to different cortisone concentrations. (n = 24)


LIFE SPAN (days)
5.6
5.5
5.4
5.3
5.2
5.1
5
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4
0
0.2
0.4
0.6
0.8
1
(Thousands)
CORTISONE CONCENTRATION (micromolar)

uM and then decreases as cortisone concentration is increased. The lifespan increase occurs over a significant range from 10 to 200 uM.

The reproductive profile is found in Table 14. The prereproductive and reproductive times were significantly altered by cortisone. At a cortisone concentration of 200 uM, the length of the prereproductive period ($F_{6, 161} = 4.826$, $p \leq 0.05$) of the rotifer increased significantly (3.24 ± 0.15 days) when compared to the control (2.63 ± 0.17 days). The length of the reproductive stage ($F_{6, 161} = 7.248$, $p \leq 0.01$) also increased significantly at 200 uM of cortisone (3.00 ± 0.29 days) ; the value of the control was 2.33 ± 0.19 days. The cortisone concentrations of 100 and 200 uM caused a significant increase in offspring number ($F_{6, 161} = 6.971$, $p \leq 0.01$) (100uM; 8.00 ± 0.90 and 200uM; 9.83 ± 0.78) when compared to the control (6.33 ± 0.48).

Figure 19 emphasizes the increase in lifespan by displaying each stage of the reproductive profile as stacked-bar graphs (stacked histograms). The reproductive stages for each cortisone concentration used are displayed individually in the form of a stacked histogram. The histograms of the rotifers treated with 100 and and 200 uM cortisone show that the increase in

Table 14

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT CORTISONE CONCENTRATIONS

Concentr. (uM)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Offspring Number/ Rotifer
0	2.63 ± 0.17	2.33 ± 0.19	0.42 ± 0.11	6.33 ±0.48
10	2.79 ± 0.11	2.25 ± 0.58	0.38 ± 0.13	7.00 ±0.65
50	2.82 ± 0.10	2.53 ± 0.36	0.15 ± 0.07	7.00 ±0.86
100	3.13 ± 0.08	2.75 ± 0.49	0.62 ± 0.14	8.00 ±0.90*
200	3.24 ± 0.15*	3.00 ± 0.29*	0.18 ± 0.06	9.83 ±0.78*
400	2.50 ± 0.17	2.04 ± 0.38	0.67 ± 0.15	5.33 ±0.41
800	2.38 ± 0.09	2.00 ± 0.29	0.66 ± 0.13	5.83 ±0.60

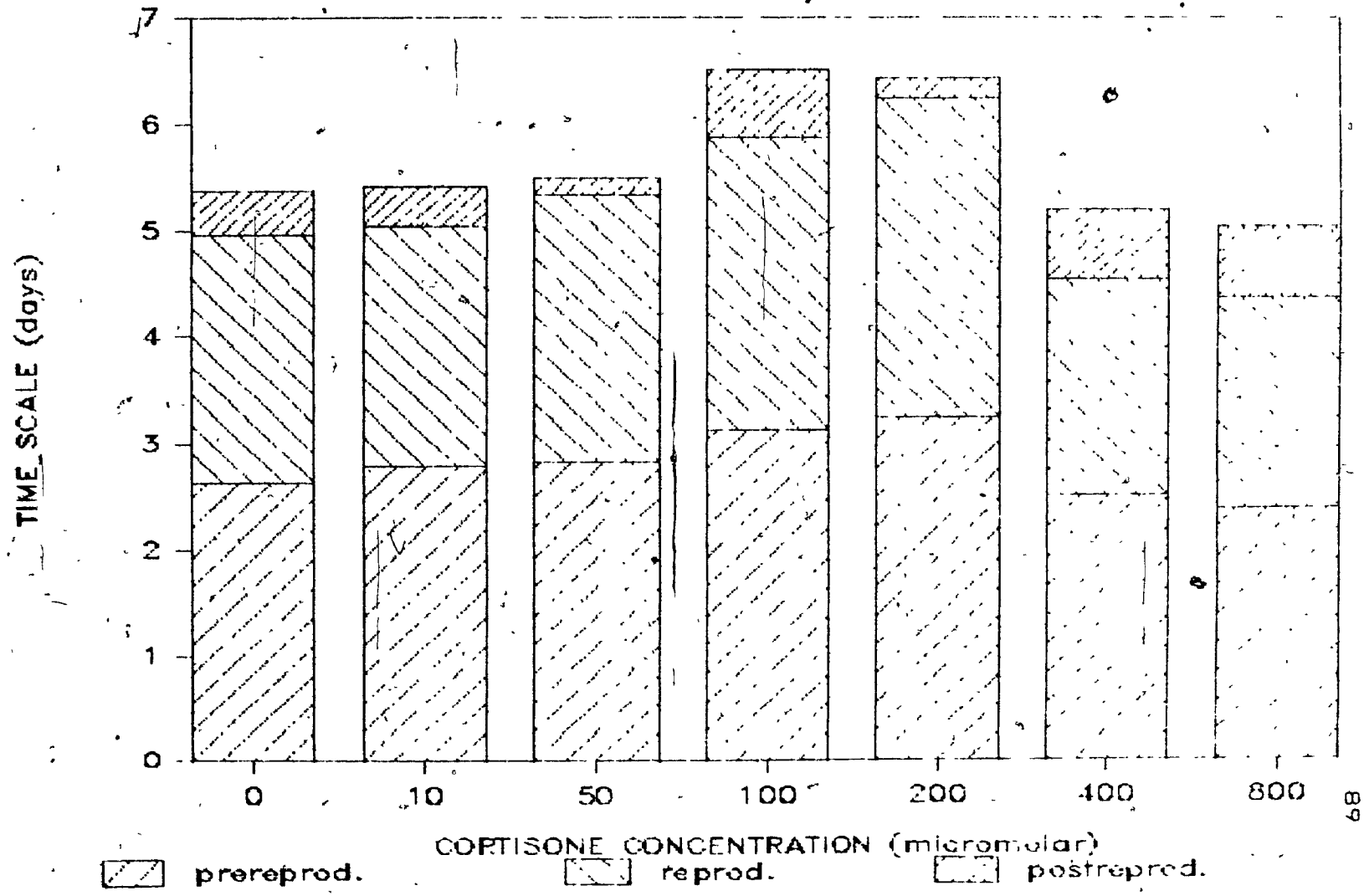
* Statistically significantly different from the control. alpha = 0.01

Figure 19. Life-profiles of A. brightwelli exposed to different cortisone concentrations are expressed as histograms (stacked-bar graphs). (n = 24)

prereprod. = prereproductive period

reprod. = reproductive period

postreprod. = postreproductive period


TIME SCALE (days)
0
1
2
3
4
5
6
7
0
10
50
100
200
400
800
89
CORTISONE CONCENTRATION (micromolar)
prereprod.
reprod.
postreprod.

lifespan of the rotifers is caused by an increase in the prereproductive and reproductive period.

Body Size of Rotifers

Since the lifespan of the rotifer was increased at cortisone concentrations of 100 and 200 uM, further experiments were performed using these optimum cortisone concentrations to explain the lifespan increase.

In this section, the body length and width of the rotifer was examined to determine whether the lifespan increase was due to a change in growth rate.

The body length and width of control rotifers and rotifers exposed to cortisone concentrations from 10 to 200 uM were measured from day 1 to day 6. The results obtained are shown in Table 15 and 16. Table 15 indicates that from day 1 to day 6, rotifers exposed to 100 and 200 uM of cortisone are all shorter in length than the control; this difference is statistically significant. For example, on day 1 ($F_{4, 115} = 14.824$, $p \leq 0.01$) the control rotifers have a mean body length of 428.3 $\pm$ 5.8 um, whereas rotifers treated with 100 uM cortisone have a length of 385.4 $\pm$ 5.0 um and rotifers exposed to 200 uM cortisone are 386.5 $\pm$ 6.7 um in length.

Table 15

THE EFFECT OF CORTISONE ON BODY LENGTH OF A. BRIGHTWELLI
(N = 24)

Age (days)	Control ± S.E. (um)	10 uM cortisone sol'n ± S.E. (um)	50 uM cortisone sol'n ± S.E. (um)
1	428.3 ± 5.8	429.1 ± 6.3	422.9 ± 5.0
2	526.0 ± 4.4	524.0 ± 4.1	513.5 ± 3.7
3	568.8 ± 2.3	571.9 ± 1.7	552.1 ± 4.0*
4	632.3 ± 3.2	627.1 ± 3.0	623.1 ± 2.8
5	709.3 ± 2.9	708.3 ± 3.0	712.5 ± 2.5
6	752.1 ± 3.3	745.8 ± 3.6	756.1 ± 2.8

* Statistically significantly different from the control.
alpha = 0.01

Age (days)	Control + S.E. (um)	100 uM cortisone sol'n + S.E. (um)	200 uM cortisone sol'n + S.E. (um)
1	428.3 ± 5.8	385.4 ± 5.0*	386.5 ± 6.7*
2	526.0 ± 4.4	466.7 ± 2.5*	465.6 ± 3.3*
3	568.8 ± 2.3	516.7 ± 3.6*	519.8 ± 4.0*
4	632.3 ± 3.2	552.1 ± 3.3*	543.8 ± 3.8*
5	709.3 ± 2.9	632.3 ± 2.8*	629.2 ± 2.5*
6	752.1 ± 3.3	708.8 ± 3.4*	709.4 ± 2.9*

* Statistically significantly different from the control.
alpha = 0.01

Three day old rotifers exposed to 50 uM cortisone were also significantly shorter in length (552.1 ± 4.0 um) as compared to the control (568.8 ± 2.3 um).

Figure 20 gives a graphic representation as to the effect of cortisone on rotifer body length. The graphs representing the 100 and 200 uM concentrations are further to the left than the control and 50 and 100 uM graphs, indicating that at 100 and 200 uM cortisone the length of the rotifer is reduced.

As is indicated in Table 16 the body width of rotifers treated with 100 and 200 uM was also significantly decreased ($(F 4, 115) = 9.344$, $p \leq 0.01$ for day 1) on all days as compared to the control.

Figure 21 consists of the body width graph; the control graph and the graphs of 10 and 50 uM concentrations all lie within the same area, whereas the graphs of the 100 and 200 uM concentrations are shifted further to the left, showing that the body width is reduced.

Lysosomes

A fluorescent vital stain specific for lysosomes was added to day 1 - day 6 old control rotifers and cortisone treated rotifers to examine whether the

Figure 20. Body length of A. brightwelli exposed to different cortisone concentrations as compared to control. (n = 24)

control = 0 cortisone concentration

10 uM = 10 micromolar cortisone concentration

50 uM = 50 micromolar cortisone concentration

100 uM = 100 micromolar cortisone concentration

200 uM = 200 micromolar cortisone concentration

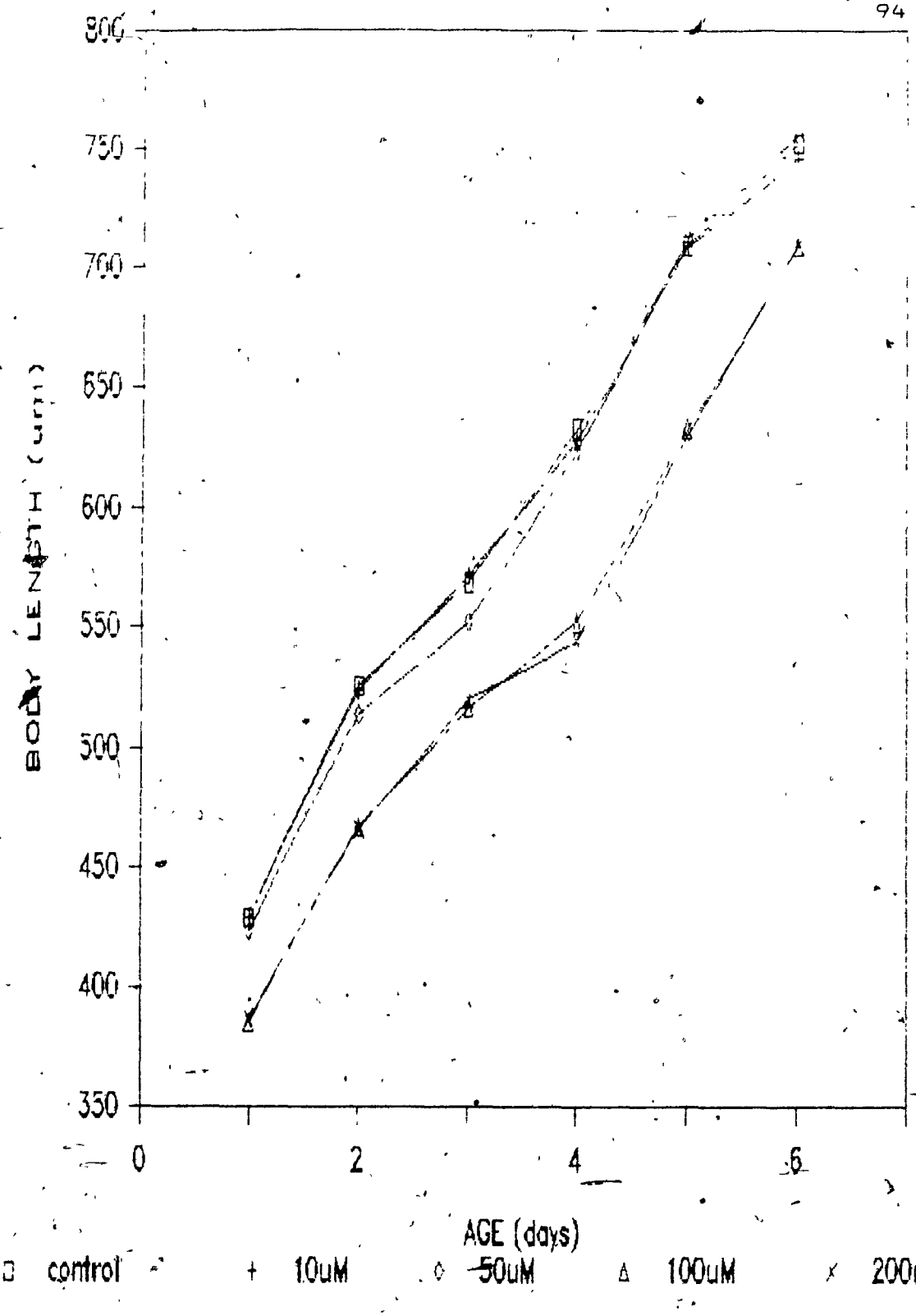

800
750
700
650
600
550
500
450
400
350
0
2
4
6
AGE (days)
control
10uM
50uM
100uM
200uM

Table 16

THE EFFECT OF CORTISONE ON BODY WIDTH OF A. BRIGHTWELLI
(N = 24)

Age (days)	Control ± S.E. (um)	10 uM cortisone sol'n ± S.E. (um)	50 uM cortisone sol'n ± S.E. (um)
1	236.5 ± 2.6	233.3 ± 3.6	228.1 ± 4.3
2	288.5 ± 7.7	280.2 ± 6.7	267.7 ± 4.4
3	295.8 ± 5.8	276.0 ± 4.4	274.0 ± 4.6
4	333.3 ± 2.5	337.5 ± 2.6	304.2 ± 6.3
5	361.5 ± 2.6	360.4 ± 2.6	358.9 ± 8.5
6	418.8 ± 5.0	425.0 ± 2.6	414.3 ± 3.8

-No significant difference between any group.

Age (days)	Control ± S.E. (um)	100 uM cortisone sol'n ± S.E. (um)	200 uM cortisone sol'n ± S.E. (um)
1	236.5 ± 2.6	209.4 ± 4.5*	212.5 ± 4.8*
2	288.5 ± 7.7	241.7 ± 3.3*	243.8 ± 3.8*
3	295.8 ± 5.8	264.6 ± 4.5*	262.5 ± 5.4*
4	333.3 ± 2.5	280.2 ± 4.5*	281.3 ± 5.0*
5	361.5 ± 2.6	321.9 ± 5.5*	329.2 ± 4.2*
6	418.8 ± 5.0	355.2 ± 3.7*	363.5 ± 4.3*

* Statistically significantly different from the control.
alpha = 0.01

Figure 21. Body width of A. brightwelli exposed to different cortisone concentrations as compared to control. (n = 24)

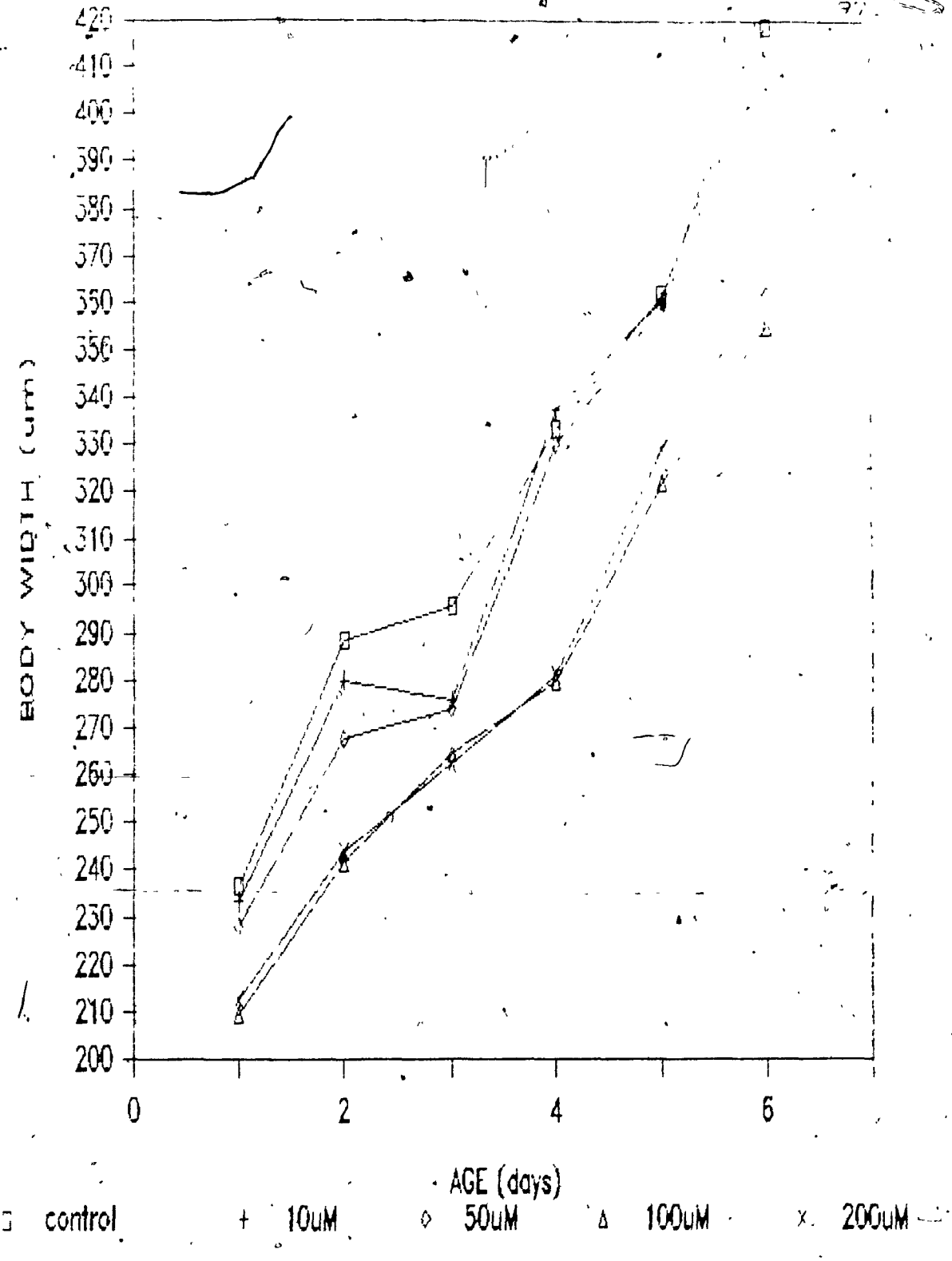
control = 0 cortisone concentration

10 uM = 10 micromolar cortisone concentration

50 uM = 50 micromolar cortisone concentration

100 uM = 100 micromolar cortisone concentration

200 uM = 200 micromolar cortisone concentration


BODY WIDTH (um)
420
410
400
390
380
370
360
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
0
2
4
6
AGE (days)
control
10uM
50uM
100uM
200uM

lifespan increase was related to a change in lysosome accumulation or activity.

The results are found in Table 17 and show that the control rotifers and those exposed to cortisone concentrations of 10 and 50 uM, exhibit fluorescence starting at day 2, indicating the presence of lysosomes.

In contrast, rotifers treated with 100 and 200 uM cortisone did not exhbit fluorescence at any age, revealing that lysosomes were either absent, physiologically altered, or present in minimal amounts as a result of cortisone treatment.

Hydrocortisone

Longevity

The influence of the hydrocortisone concentrations, ranging from 10 to 800 uM, on rotifer lifespan as compared to the control are introduced in Table 18. The results show that the rotifers exposed to a 100 uM hydrocortisone solution have a significantly longer lifespan than any other group. The results also show that hydrocortisone concentrations of 200, 400 and 800 uM, greatly shorten the rotifer lifespan. A one-way analysis of variance confirmed that significant

Table 17

THE EFFECT OF CORTISONE ON LYSOSOME PRESENCE IN THE ROTIFER A. BRIGHTWELLI
(lysosome detection via fluorescent vital stain)
(N = 12)

Concentration of cortisone (uM)	Amount of fluorescence=presence of lysosomes					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
0	-	+	+	+	+	+
10	-	+	+	+	+	+
50	-	+	+	+	+	+
100	-	-	-	-	-	-
200	-	-	-	-	-	-

\+ fluorescence

\- no fluorescence

Table 18

THE EFFECT OF HYDROCORTISONE ON THE LIFE-SPAN OF A. BRIGHTWELL-I

(N = 24)

Concentration of Hydrocortisone solution (micromolar)	Maximum Longevity (Days)	Mean Lifespan ± S.E.M. (Days)
0	6.50	5.54 ± 0.10
10	7.50	5.57 ± 0.31
50	7.00	5.25 ± 0.25
100	8.25	6.25 ± 0.18*
200	2.50	2.08 ± 0.09*
400	1.00	0.92 ± 0.08*
800	1.00	0.79 ± 0.13*

*Statistically significantly different from the control. alpha = 0.01

differences occured among the lifespan of the groups ((F 6, 161) = 10.238, $p \leq 0.01$). The mean lifespan was significantly increased at 100 uM hydrocortisone concentration (6.25 $\pm$ 0.18 days) and decreased at concentrations of 200 uM (2.08 $\pm$ 0.09 days), 400 uM (0.92 $\pm$ 0.08 days) and 800 uM (0.79 $\pm$ 0.13 days). The mean lifespan of the control was 5.54 $\pm$ 0.10 days.

Figure 22 presents the survival curves of the rotifers treated with the four lowest levels of hydrocortisone and of the control. As can be seen in Figure 22, the survival curve of the rotifers treated with 100 uM hydrocortisone lies furthest to the right, pointing out that the longest lifespan occured at this concentration. Figure 23 shows the survival curves for the rotifers treated with the two highest concentrations (400 and 800 uM). These curves are placed far to the left, showing that lifespan is much shorter at these concentrations. This is also true for rotifers treated with 200 uM hydrocortisone.

In Figure 24, mean lifespan is plotted against the increasing concentrations of hydrocortisone to which the groups of rotifers were exposed. Figure 24 indicates that lifespan first increases, peaking at 100 uM, followed by a drastic and continuous decrease as

Figure 22. Survivorship curves of A. wrightwelli exposed to the lower range of hydrocortisone concentrations as compared to control. (n = 24)

control = 0 hydrocortisone concentration

10 uM = 10 micromolar hydrocortisone concentration

50 uM = 50 micromolar hydrocortisone concentration

100 uM = 100 micromolar hydrocortisone concentration

200 uM = 200 micromolar hydrocortisone concentration

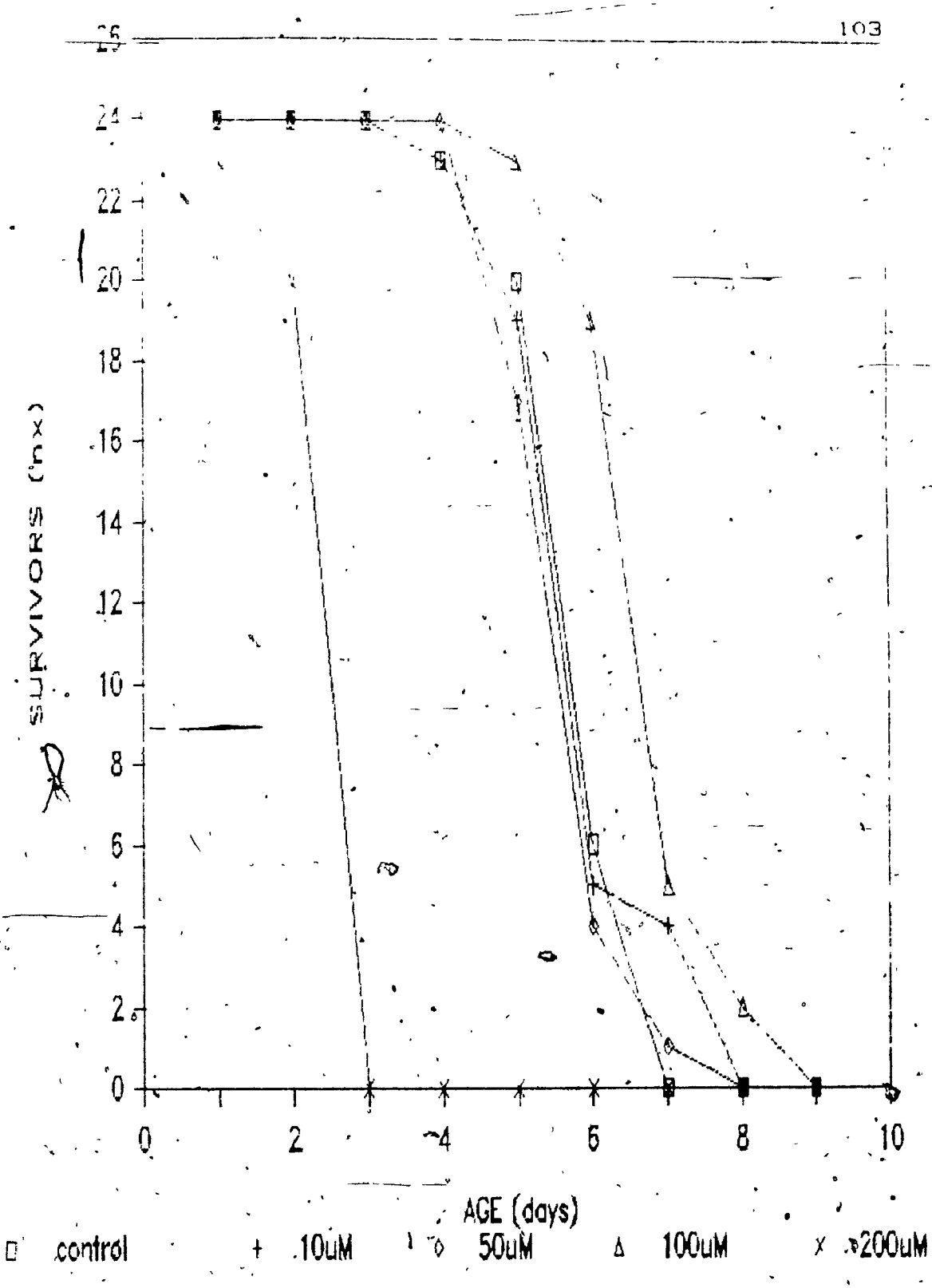

SURVIVORS (n x)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
AGE (days)
control
10uM
50uM
100uM
200uM

Figure 23. Survivorship curves of A. brightwelli exposed to the higher range of hydrocortisone concentrations as compared to control. (n = 24)

control = 0 hydrocortisone concentration

400 uM = 400 micromolar hydrocortisone concentration

800 uM = 800 micromolar hydrocortisone concentration

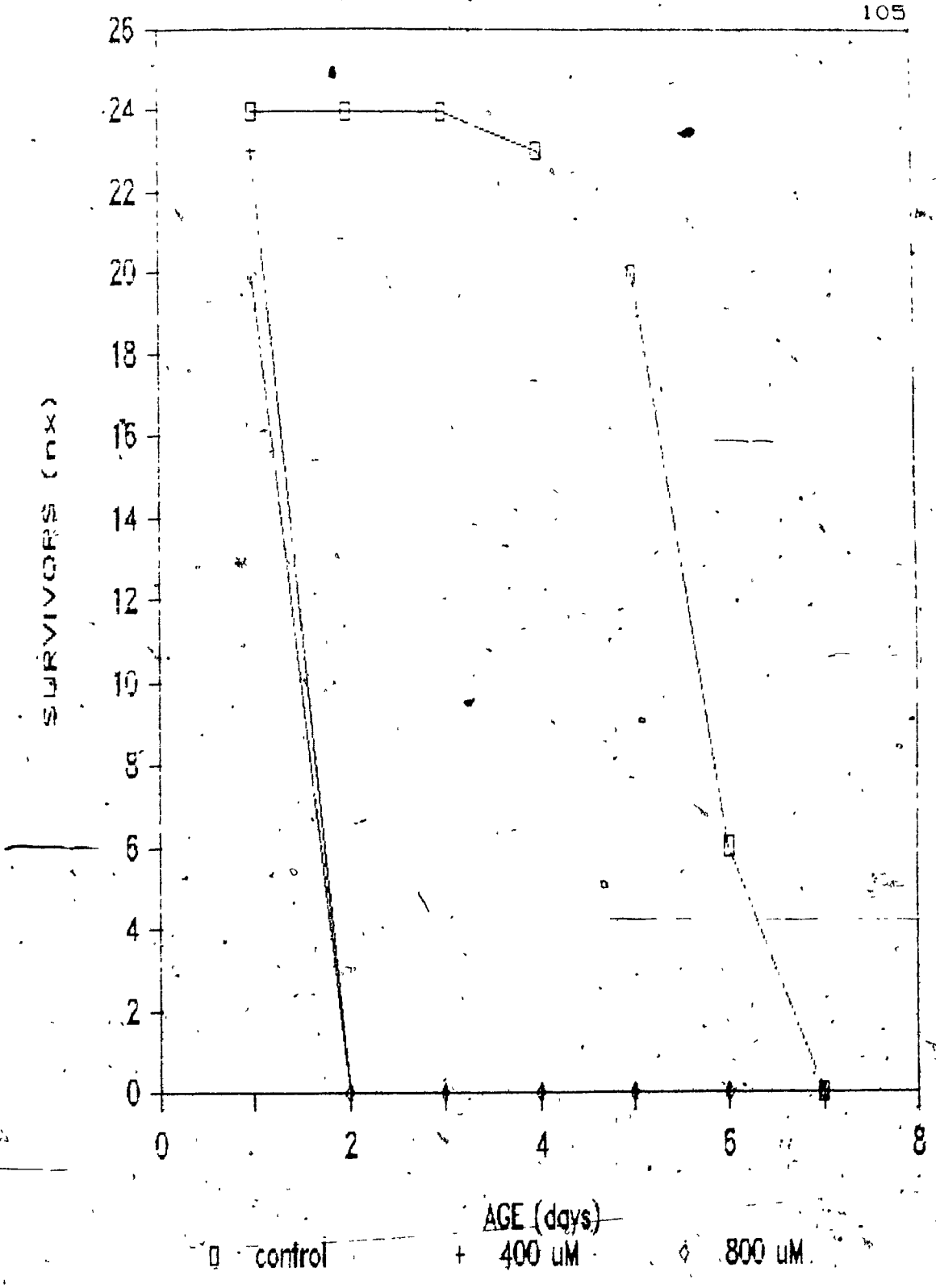
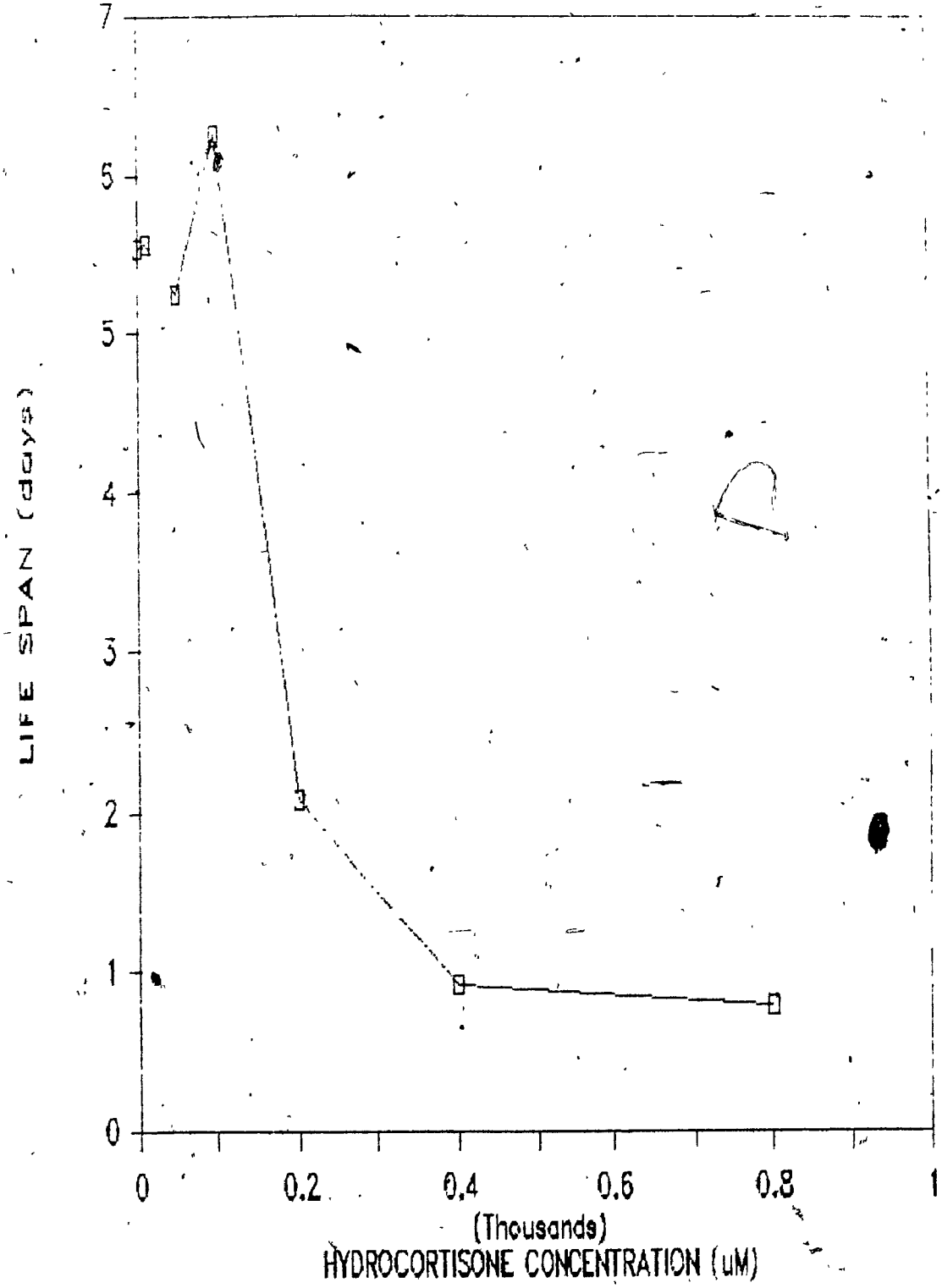

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
400 uM
800 uM

Figure 24. Average life-span versus hydrocortisone concentration graph of A. brightwelli exposed to different hydrocortisone concentrations. (n = 24)


LIFE SPAN (days)
7
5
5
4
3
2
1
0
0
0.2
0.4
0.6
0.8
1
(Thousands)
HYDROCORTISONE CONCENTRATION (uM)

hydrocortisone concentration is is increased.

Table 19 contains the reproductive profile for rotifers treated with hydrocortisone. The 10 uM hydrocortisone concentration has no effect on the reproductive profile. At 50 uM (1.85 ± 0.18 days) and at 100 uM (1.95 ± 0.25 days) both the length of the reproductive period ($(F\ 6,161) = 10.893$, $p \leq 0.01$) and the number of offspring ($(F\ 6, 161) = 12.725$, $p \leq 0.01$) a significantly reduced. It is only at 100 uM hydrocortisone (3.59 ± 0.06 days) that the length of the prereproductive period ($(F\ 6, 161) = 9.684$, $p \leq 0.01$) is significantly increased , resulting in increased mean lifespan; the control had a prereproductive period of 2.53 ± 0.07 days. At the higher concentrations of hydrocortisone toxic effects appear, including a significant reduction prereproductive period; at 400 uM this period lasted for 0.92 ± 0.09 days and at 800 uM hydrocortisone, it was 0.79 ± 0.13 days long in comparison to 2.53 ± 0.07 days for the control. The reproductive and postreproductive time ($(F\ 6, 35) = 20.459$, $p \leq 0.01$) plus offspring number was 0 at 400 and 800 uM hydrocortisone concentrations.

Figure 25 emphasizes the increase in lifespan by displaying each stage of the reproductive profile as

Table 19

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT HYDROCORTISONE CONCENTRATIONS

Concentr. (uM)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Ave. Offspring Number/ Rotifer
0	2.53 ± 0.07	2.63 ± 0.25	0.38 ± 0.08	8.00 ±0.41
10	3.10 ± 0.17	2.03 ± 0.13	0.47 ± 0.09	7.50 ±0.52
50	2.85 ± 0.12	1.85 ± 0.18*	0.55 ± 0.07	5.83 ±0.67*
100	3.59 ± 0.06*	1.95 ± 0.25*	0.71 ± 0.10	5.33 ±0.32*
200	2.08 ± 0.08	0	0	0
400	0.92 ± 0.09	0	0	0
800	0.79 ± 0.13	0	0	0

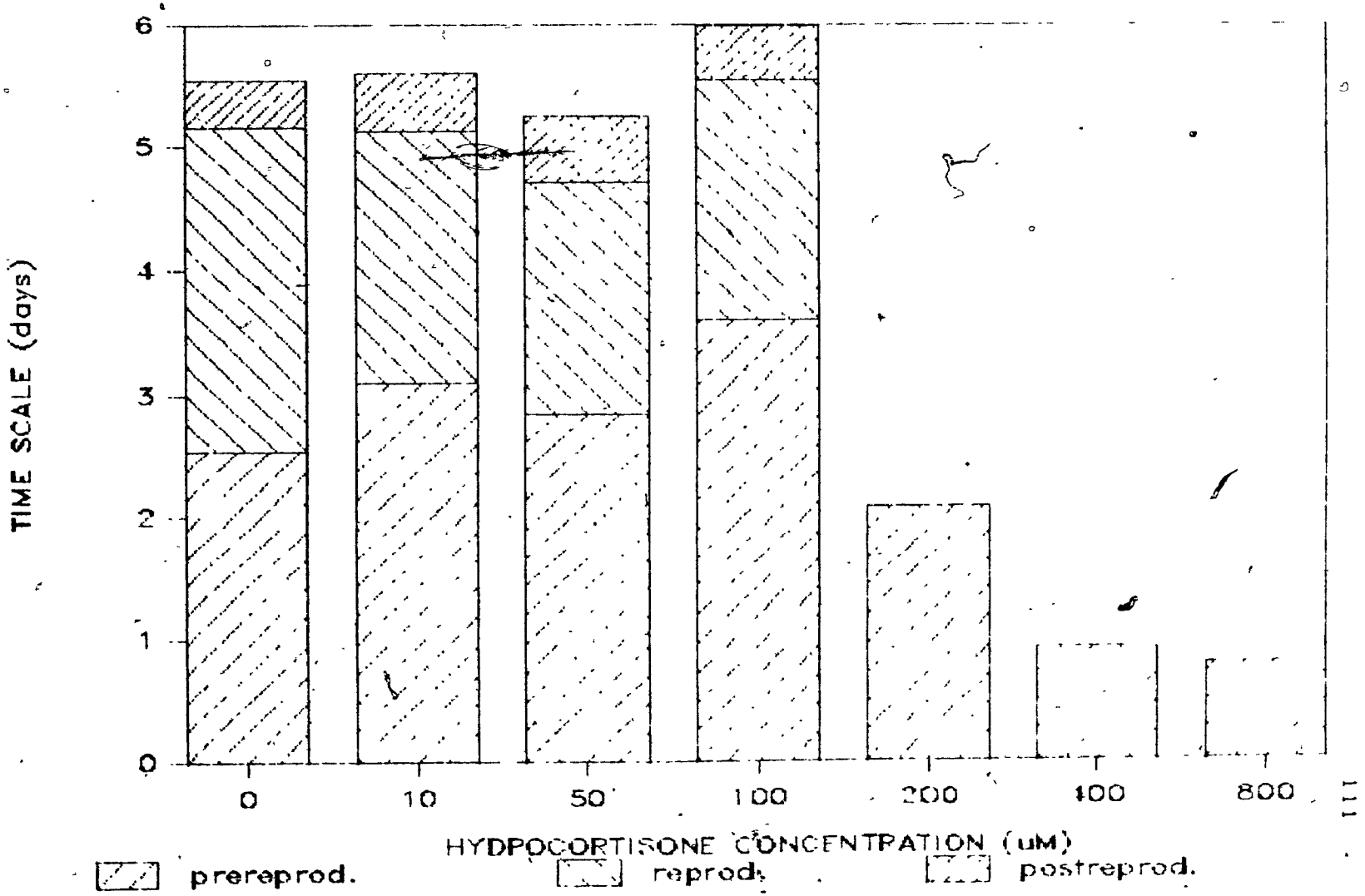
* Statistically significantly different from the control.
alpha = 0.01

Figure 25. Life profiles of A. brightwelli exposed to different hydrocortisone concentrations are expressed as histograms (stacked-bar graphs). (n = 24)

prereprod. = prereproductive period

reprod. = reproductive period

postreprod. = postreproductive period


TIME SCALE (days)
0
1
2
3
4
5
6
0
10
50
100
200
400
800
HYDPOCORTISONE CONCENTRATION (uM)
prereprod.
reprod.
postreprod.

stacked-bar graphs; each histogram shows the reproductive stages for a specific hydrocortisone concentration. The histograms indicates that the increase in lifespan of rotifers treated with 100 uM is caused by an increase in the prereproductive period and that the decrease in lifespan of rotifers treated at higher hydrocortisone concentrations is due to a decrease in prereproductive period and a total absence of the reproductive and postreproductive period.

Body Size of Rotifers

Since the lifespan of the rotifer was increased at a hydrocortisone concentration of 100 uM, the same follow-up experiments were done as for cortisone. This optimum hydrocortisone concentration was used to explain the lifespan increase.

In this section, the body length and width of the rotifer were measured to determine if the lifespan increase was due to a change in growth rate.

The body length and width of control rotifers and rotifers exposed to hydrocortisone concentrations ranging from 10 to 100 uM were measured from day 1 to day 6. The data for body length is displayed in Table 20. This table shows that rotifers exposed to 100 uM

Table 20

THE EFFECT OF HYDROCORTISONE ON BODY LENGTH OF A. BRIGHTWELLI
(N = 24)

Age (days)	Control + S.E. (um)	10 uM hydrocort. sol'n + S.E. (um)	50 uM hydrocort. sol'n + S.E. (um)	100 uM hydrocort. sol'n+S.E. (um)
1	411.7 + 9.4	417.3 + 10.7	410.9 + 9.4	376.0 + 9.6*
2	534.4 + 13.5	482.3 + 8.3**	480.5 + 6.4**	380.8 +10.2*
3	572.5 + 10.1	562.5 + 10.2	558.2 + 7.0	468.3 + 6.9*
4	653.2 + 7.2	628.2 + 8.7	625.3 + 9.2	518.8 + 9.4*
5	715.6 + 7.6	701.0 + 8.7	699.5 + 6.5	618.8 + 8.1*
6	739.6 + 6.4	728.1 + 7.3	714.6 + 7.4*	700.0 + 6.4*

* Statistically significantly different from the control at alpha = 0.01

** Statistically significantly different from the control at alpha = 0.05

hydrocortisone were significantly shorter in length than the control from day 1 to day 6.

For example, on day 2 ($F_{3, 92} = 41.078$, $p \leq 0.01$) rotifers treated with 100 uM hydrocortisone had a length of 380.8 $\pm$ 9.4 um as compared to a length of 534.4 $\pm$ 13.5 um for the control. At 10 uM hydrocortisone (482.3 $\pm$ 8.3 um) body length was significantly decreased as compared to the control on day 2 only. Also only on day 2, the length of rotifers exposed to 50 uM hydrocortisone (480.5 $\pm$ 6.4 um) was shorter than the control. Figure 26 displays length versus age graphs and shows that for each hydrocortisone concentration used the length of the rotifer increases as it gets older. Yet in comparison to the control, the length of hydrocortisone-treated rotifers is much smaller at all ages, this difference is especially significant with rotifers exposed to 100 uM hydrocortisone. The 100 uM hydrocortisone graph is set further left than the control graph, indicating that at this concentration the rotifers are smaller in length than the control.

Table 21 displays the data for body width of rotifers at different hydrocortisone concentrations. The data indicates that the rotifers treated with 100 uM hydrocortisone have a significantly smaller width than

Figure 26. Body length of A. brightwelli exposed to different hydrocortisone concentrations as compared to control. (n = 24)

control = 0 hydrocortisone concentration

10 uM = 10 micromolar hydrocortisone concentration

50 uM = 50 micromolar hydrocortisone concentration

100 uM = 100 micromolar hydrocortisone concentration

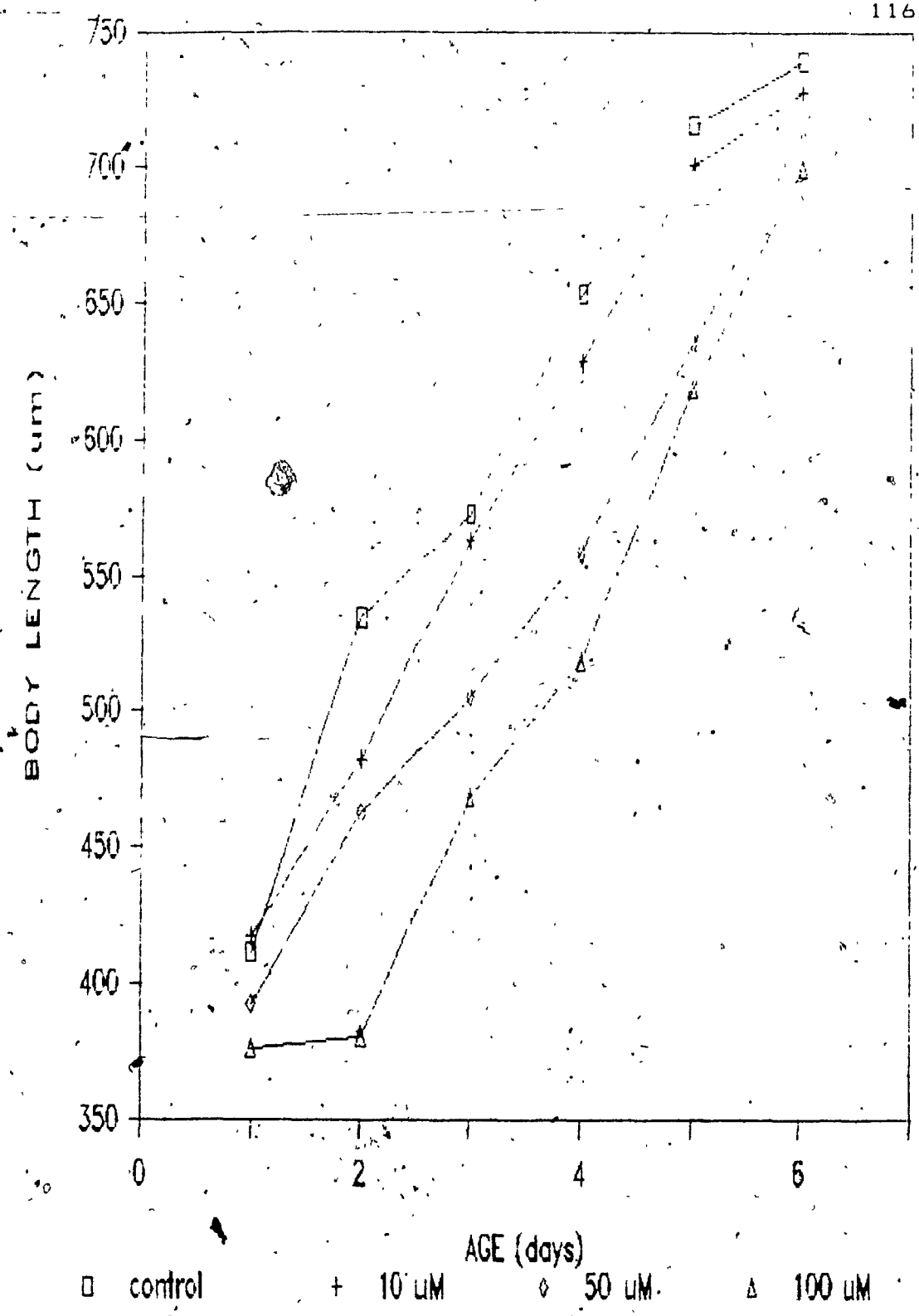

750
700
650
600
550
500
450
400
350
BODY LENGTH (um)
0
2
4
6
AGE (days)
control
10 uM
50 uM
100 uM

Table 21

THE EFFECT OF HYDROCORTISONE ON BODY WIDTH OF A. BRIGHTWELLI
(N = 24)

Age (days)	Control + S.E. (um)	10 uM hydrocort. sol'n + S.E. (um)	50 uM hydrocort. sol'n + S.E. (um)	100 uM hydrocort. sol'n+S.E. (um)
1	234.4 + 3.6	225.0 + 6.0	224.6 + 5.6	212.5 + 6.8*
2	289.6 + 9.1	266.7 + 8.1	265.2 + 4.5	239.6 + 5.6*
3	293.8 + 6.9	290.6 + 7.0	284.0 + 4.4	275.0 + 5.9*
4	346.9 + 4.3	333.3 + 7.2	327.9 + 6.4	287.5 + 6.4*
5	383.3 + 6.0	367.7 + 4.6	365.4 + 3.3	319.8 + 6.0*
6	415.6 + 6.9	418.8 + 8.4	414.0 + 5.7	340.6 + 3.9*

* Statistically significantly different from the control.
alpha = 0.01

the control; this is true at all ages. For example, on day 2 ($F_{3, 92} = 8.844$, $p \leq 0.01$) the body width of rotifers exposed to 100 uM hydrocortisone is 239.6 ± 5.6 um in comparison to the control value of 289.6 ± 9.1 um. Figure 27 shows body width graphs for each hydrocortisone concentration used and compares it to the control graph. The graphs show how body width changes as the rotifer ages. The 100 uM graph increases less with advancing age than the control indicating that width of rotifers treated with 100 uM hydrocortisone is smaller than for the control rotifers.

Lysosomes

A fluorescent vital stain specific for lysosomes was added to control rotifers and hydrocortisone treated rotifers ranging from one day to six days of age to examine whether if the lifespan increase was due to a change in lysosome accumulation or activity.

The results are found in Table 22 and show that the control rotifers and rotifers exposed to 10 and 50 uM hydrocortisone begin to show fluorescence on day 2, indicating the presence of lysosomes. When rotifers were treated with 100 uM hydrocortisone though, these rotifers displayed no fluorescence whatsoever at any age, meaning

Figure 27. Body width of A. brightwelli exposed to different hydrocortisone concentrations as compared to control. (n = 24)

control = 0 hydrocortisone concentration

10 uM = 10 micromolar hydrocortisone concentration

50 uM = 50 micromolar hydrocortisone concentration

100 uM = 100 micromolar hydrocortisone concentration

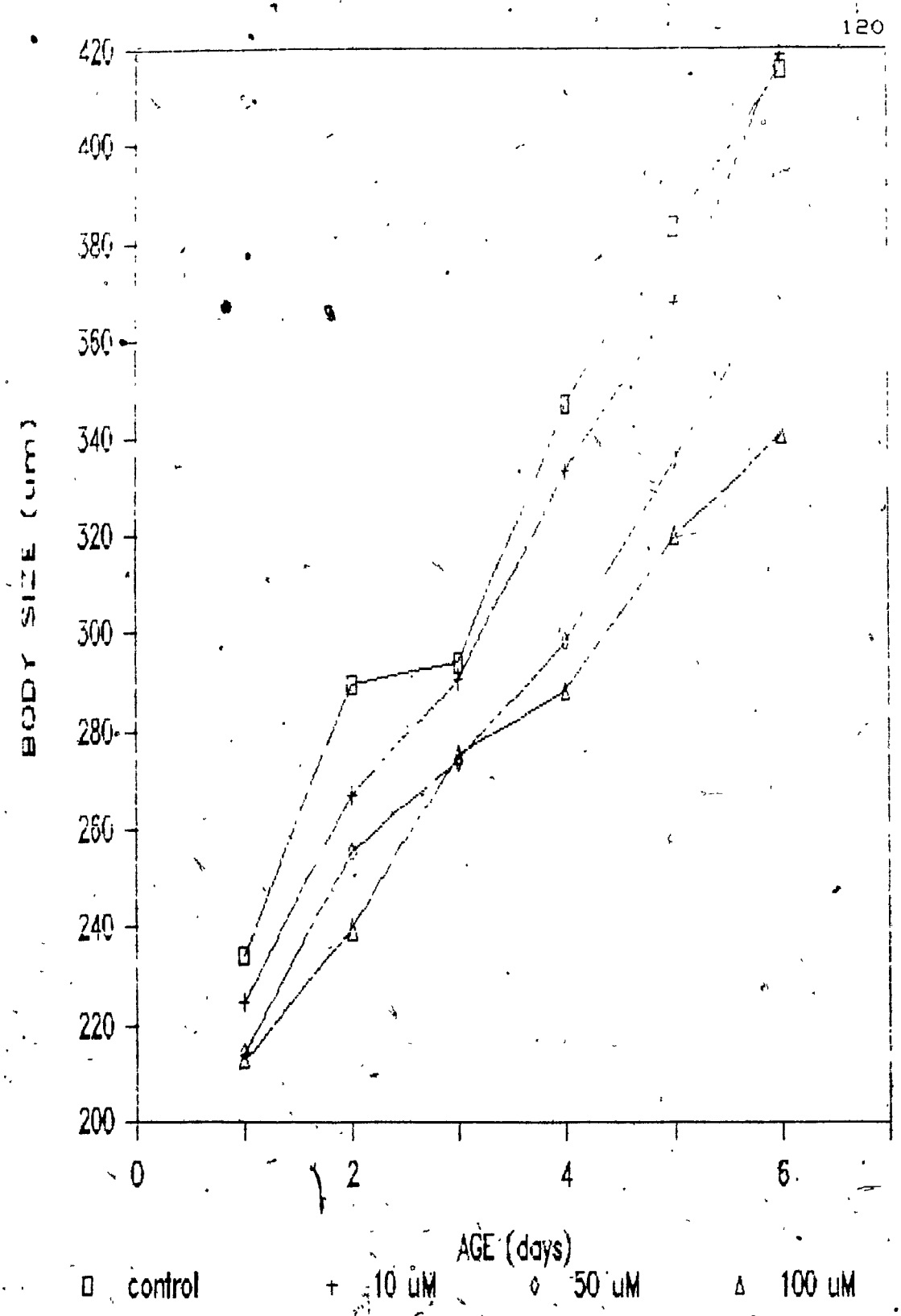

120
420
400
380
360
340
320
300
280
260
240
220
200
BODY SIZE (um)
0
2
4
6
AGE (days)
control
10 uM
50 uM
100 uM

Table 22

THE EFFECT OF HYDROCORTISONE ON LYSOSOME PRESENCE IN THE ROTIFER A. BRIGHTWELLI
(lysosome detection via fluorescent vital stain)
(N = 12)

Concentration of hydrocortisone (uM)	Amount of fluorescence=presence of lysosome					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
0	±	+	+	+	+	+
10	−	+	+	+	+	+
50	−	+	+	+	+	+
100	−	−	−	−	−	−

\+ fluorescence

− no fluorescence

that lysosomes were absent, physiologically altered or present in minimal amount at this concentration.

Ethanol

Longevity

All of the hormones used were dissolved in ethanol, therefore it was necessary to set up ethanol controls as well to determine whether it affected the rotifer in any way.

For each hormone experiment done, there were ethanol controls. These controls consisted of the same concentrations as the hormones, that is to say for each hormone experiment the ethanol concentrations were 10, 50, 100, 200, 400 and 800 uM.

The longevity data for rotifers exposed to these different ethanol concentrations are shown in Tables 23, 24, 25 and 26. Table 23 displays the ethanol results obtained during the B-estradiol experiment. A one-way analysis of variance followed by a post hoc Tukey test showed that there were significant differences in lifespan between the control group and the rotifers exposed to 400 and 800 uM ethanol ($(F\ 6, 161) = 6.789$, $p \leq 0.05$); the mean lifespan of rotifers exposed to 400 (4.17

Table 23

THE EFFECT OF ETHANOL ON THE LIFE-SPAN OF A. BRIGHTWELLI
(B-estradiol experiment) (N = 24)

Concentration of Ethanol solution	Maximum Longevity (Days)	Mean Lifespan ± S.E.M. (Days)
0	6.50	5.08 ± 0.14
10	5.50	5.08 ± 0.42
50	6.00	5.42 ± 0.25
100	5.50	5.08 ± 0.15
200	5.50	5.00 ± 0.13
400	4.75	4.17 ± 0.21*
800	5.00	3.88 ± 0.70*

* Statistically significantly different from the control.
alpha = 0.05

± 0.21 days) and 800 uM (3.88 ± 0.70 days) ethanol is shorter than that of the control (5.08 ± 0.14 days). Table 24 ((F 6, 161) = 2.695, p>0.01) contains the ethanol results gathered during the thyroxine experiment; data of Table 25 ((F 6, 161) = 1.931, p>0.01) was taken during the cortisone experiment; and the results of Table 26 ((F 6, 161 = 3.357, p>0.01) during the hydrocortisone experiment.

A one-way analysis of variance followed by the post hoc Tukey test reveals that there are no significant differences in mean lifespan between the control and the different ethanol concentrations in any of these tables. despite the absence statistical differences, the mean lifespan of rotifers exposed to 400 and 800 uM of ethanol is a little shorter than the control in all tables.

The relationship between the lifespan values can also be emphasized graphically. In Figure 28 lifespan is plotted against ethanol concentration. All graphs representing the ethanol concentrations during the different hormone experiments shows that a small decrease occurs in lifespan of rotifers exposed to 400 and 800 uM ethanol concentrations. This decrease is most pronounced in the graph representing data taken during the B-estradiol experiment.

Table 24

THE EFFECT OF ETHANOL ON THE LIFE-SPAN OF A. BRIGHTWELLI
(Thyroxine experiment) (N = 24)

Concentration of Ethanol solution (micromolar)	Maximum Longevity (Days)	Mean Lifespan ± S.E.M. (Days)
0	7.50	5.71 ± 0.42
10	7.00	5.41 ± 0.23
50	7.00	5.25 ± 0.36
100	7.00	5.29 ± 0.25
200	6.50	5.00 ± 0.22
400	5.50	4.75 ± 0.19
800	5.50	4.83 ± 0.19

- No significant difference between any group. alpha =0.01

Table 25

THE EFFECT OF ETHANOL ON THE LIFE-SPAN OF A. BRIGHTWELLI
(cortisone experiment) (N = 24)

Concentration of Ethanol solution (micromolar)	Maximum Longevity (days)	Mean Lifespan ± S.E.M. (days)
0	7.0	5.38 ± 0.32
10	7.0	5.37 ± 0.11
50	7.0	5.34 ± 0.12
100	7.0	5.45 ± 0.20
200	7.0	5.29 ± 0.22
400	6.5	5.04 ± 0.18
800	5.5	4.95 ± 0.36

- No significant difference between any group. alpha = 0.01

Table 26

THE EFFECT OF ETHANOL ON THE LIFE-SPAN OF A. BRIGHTWELLI
(hydrocortisone experiment) (N = 24)

Concentration of Ethanol solution (micromolar)	Maximum Longevity (days)	Mean Lifespan ± S.E.M. (days)
0	6.50	5.54 ± 0.10
10	7.00	5.49 ± 0.28
50	7.00	5.40 ± 0.13
100	6.50	5.36 ± 0.14
200	6.50	5.30 ± 0.26
400	6.50	5.00 ± 0.32
800	5.50	4.88 ± 0.35

- No significant difference between any group. alpha = 0.01

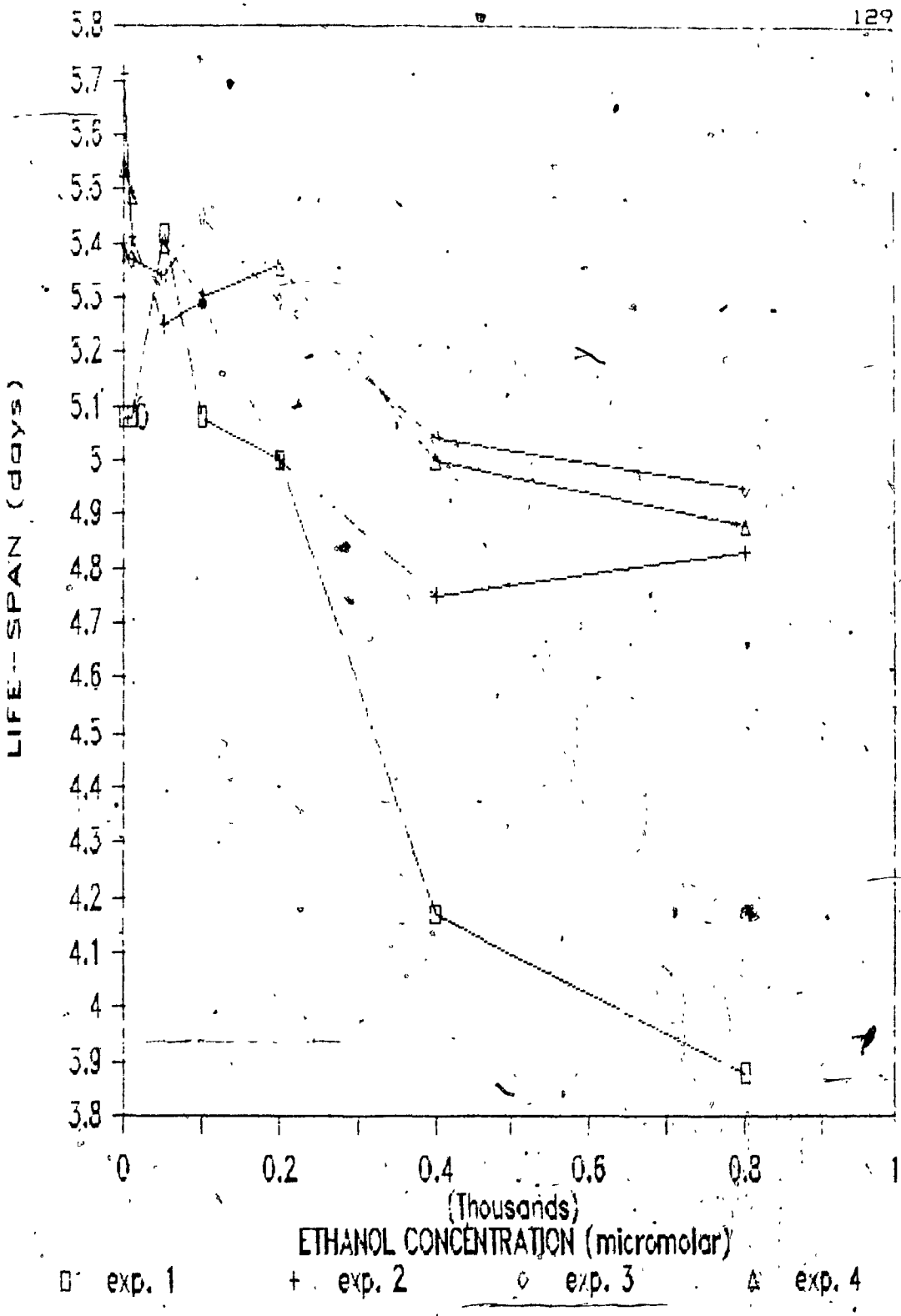
Figure 28. Life-span versus ethanol concentration of A. brightwelli exposed to different ethanol concentrations. (n = 24)

exp. 1 = ethanol results obtained during the B-estradiol experiment

exp. 2 = ethanol results obtained during the thyroxine experiment

exp. 3 = ethanol results obtained during the cortisone experiment

exp. 4 = ethanol results obtained during the hydrocortisone experiment


LIFE-SPAN (days)
5.8
5.7
5.6
5.5
5.4
5.3
5.2
5.1
5
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4
3.9
3.8
0
0.2
0.4
0.6
0.8
1
(Thousands)
ETHANOL CONCENTRATION (micromolar)
□ exp. 1
+ exp. 2
○ exp. 3
△ exp. 4

Figures 29 (B-estradiol experiment), 31 (thyroxine experiment) 33, (cortisone experiment) and 35 (hydrocortisone experiment) present the survival curves for control and rotifers treated at the lower concentrations of ethanol. All the curves in all figures lie within the same area, indicating that number of suriving rotifers is similar at ethanol concentrations ranging from 10 to 200 uM in comparison to the control. Figures 30 (B-estradiol experiment), 32 (thyroxine experiment), 34 (cortisone experiment) and 36 (hydrocortisone experiment) show the effects of higher concentrations of ethanol on rotifer survival. In all figures, the control curves lie further to the right than the survival curves for rotifers treated with 400 and 800 uM of ethanol, indicating that less rotifers survive at these two highest concentrations.

The reproductive profile is presented in Tables 27, 28, 29 and 30. A one-way analysis of variance followed by the post hoc Tukey test revealed no significant difference between any groups at any of the stages of the reproductive profile.

It is important to note that the survival curves and mean lifespan for all four replicate control groups are very close to eachother, indicating that control data is highly replicable.

Figure 29. Survivorship curves of A. brightwelli exposed to the lower range of ethanol concentrations as compared to the control. Results obtained during the B-estradiol experiment. (n = 24)

control = 0 ethanol concentration

10 uM = 10 micromolar ethanol concentration

50 uM = 50 micromolar ethanol concentration

100 uM = 100 micromolar ethanol concentration

200 uM = 200 micromolar ethanol concentration

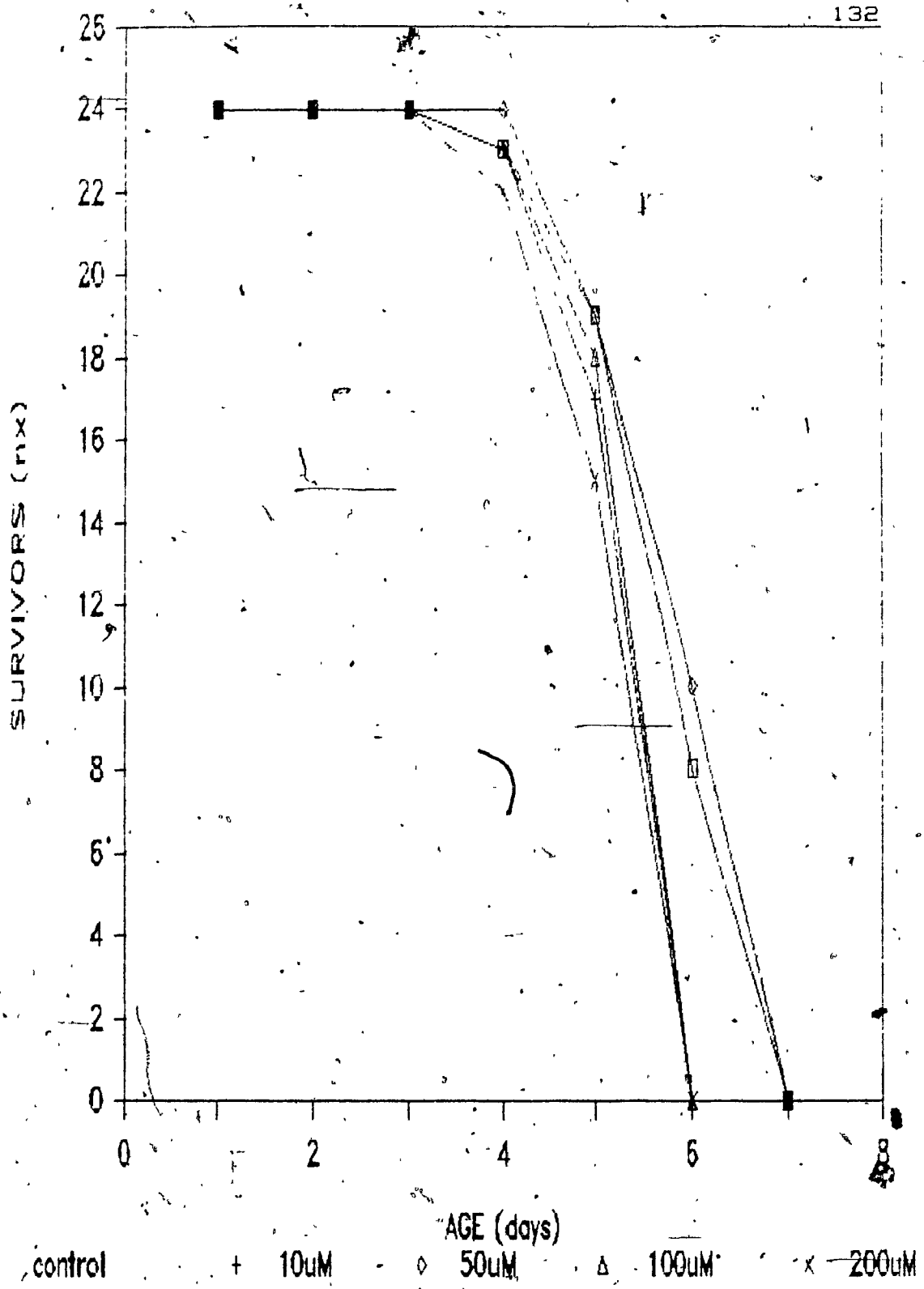

SURVIVORS (n x)
25
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
10uM
50uM
100uM
200uM

Figure 31. Survivorship curves of A. brightwelli exposed to the lower range of ethanol concentrations as compared to the control. Results obtained during the thyroxine experiment. (n = 24).

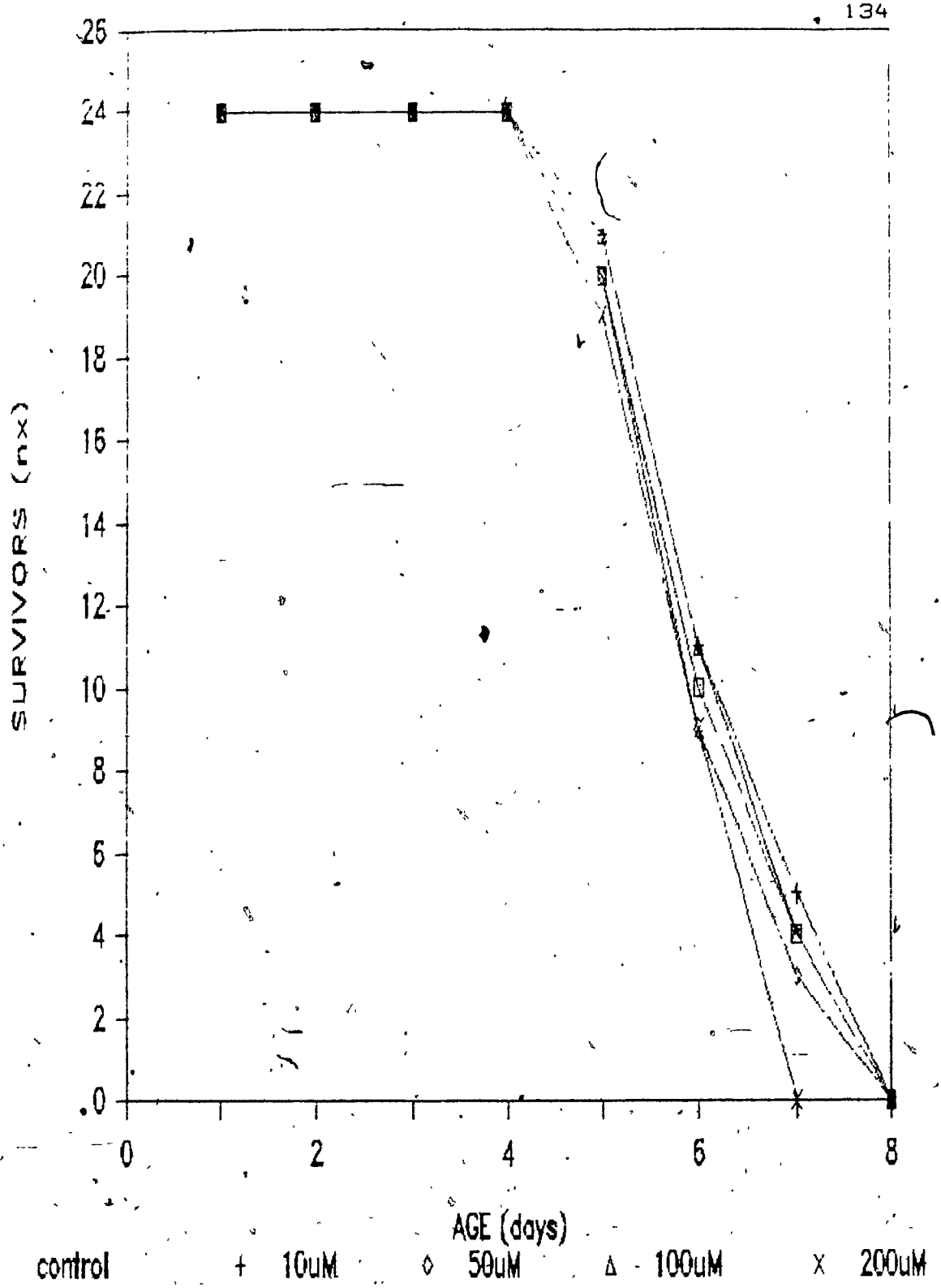

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
6
4
2
.0
0
2
4
6
8
AGE (days)
control
10uM
50uM
100uM
200uM

Figure 33. Survivorship curves of A. brightwelli exposed to the lower range of ethanol concentrations as compared to the control. Results obtained during the cortisone experiment. (n = 24)

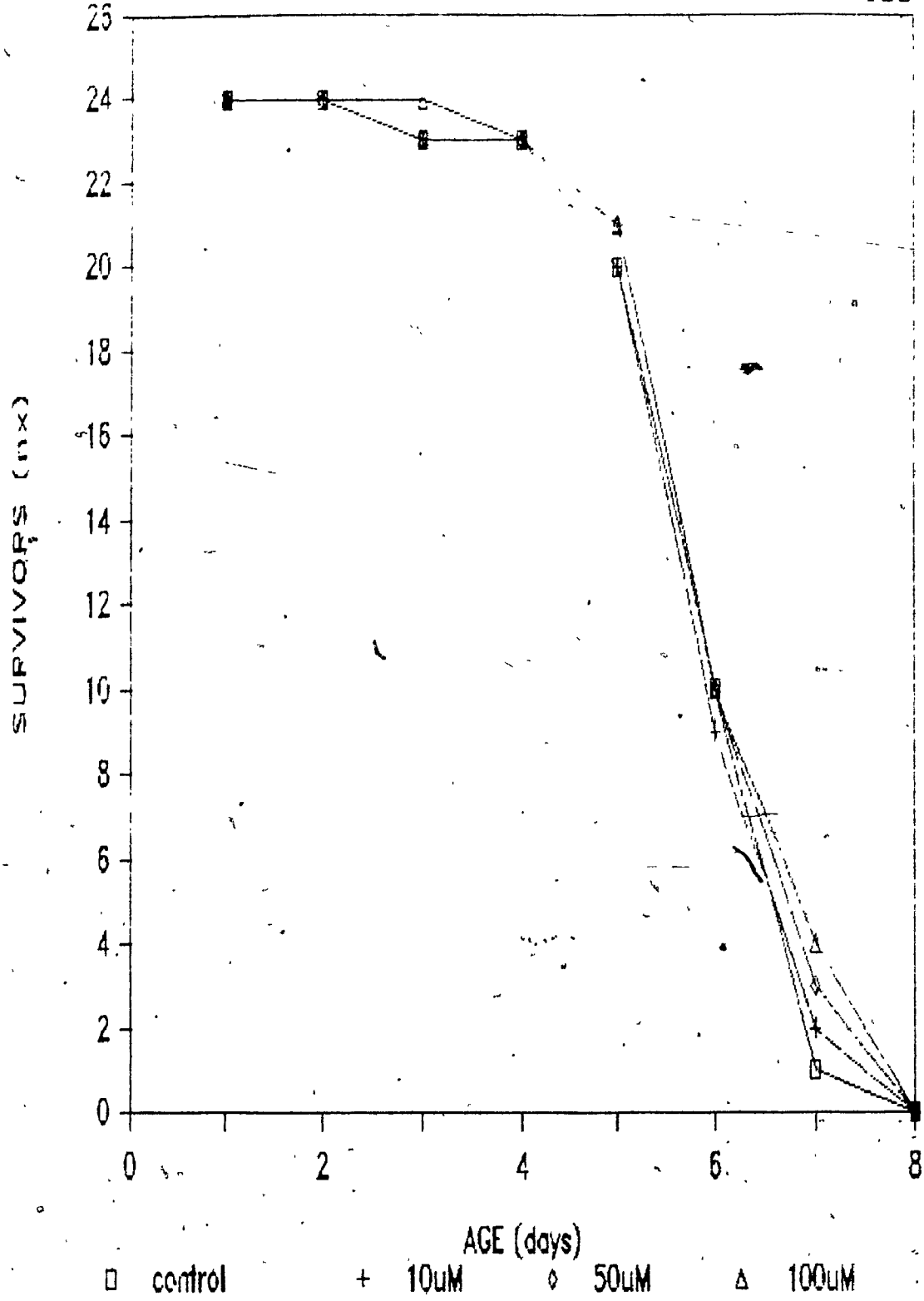

SURVIVORS (n x)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
10uM
50uM
100uM

Figure 35. Survivorship curves of A. brightwelli exposed to the lower range of ethanol concentrations as compared to the control. Results obtained during the hydrocortisone experiment. (n = 24)

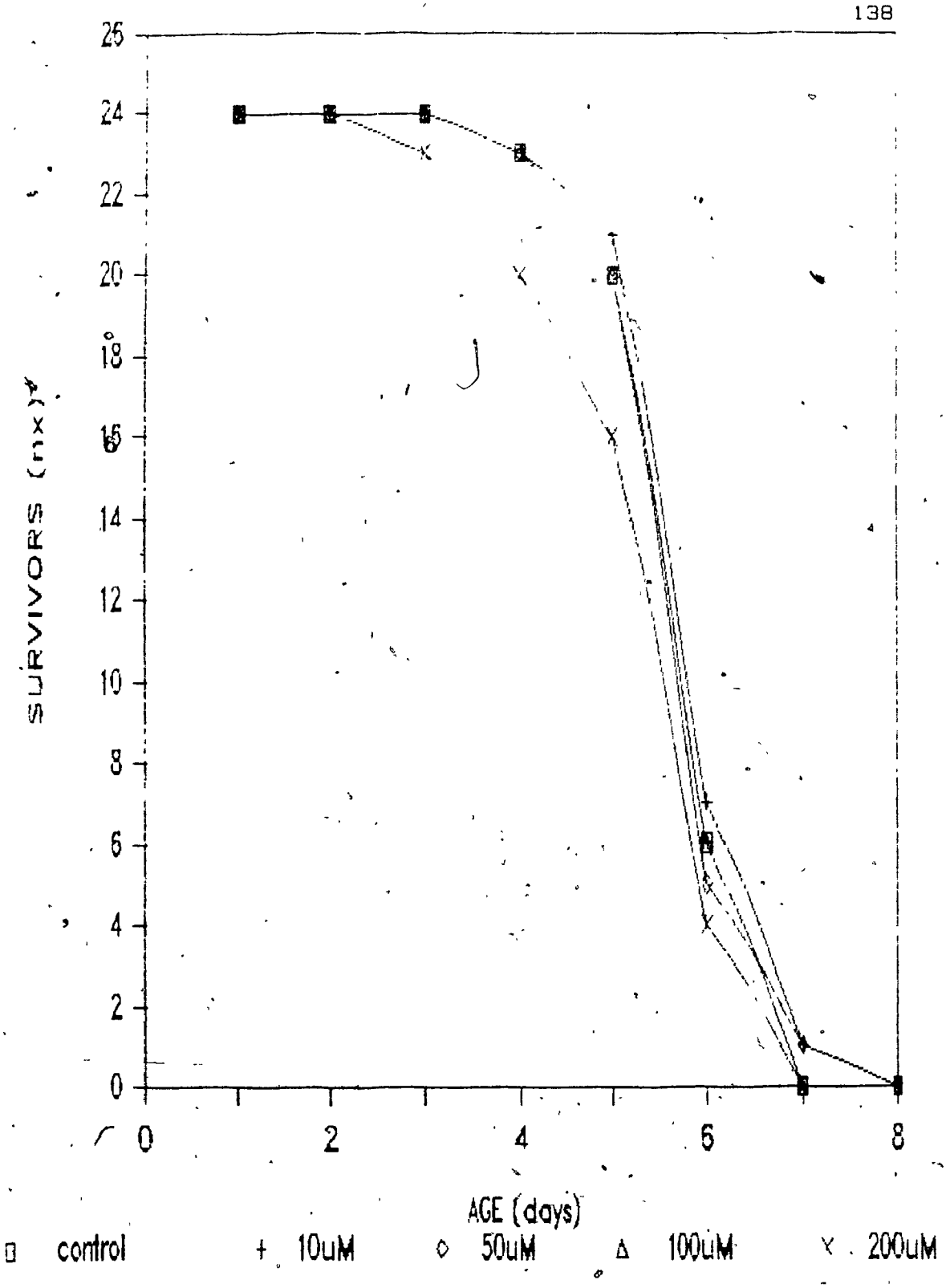

SURVIVORS (n x)
25
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
10uM
50uM
100uM
200uM

Figure 30. Survivorship curves of A. brightwelli exposed to the higher range of ethanol concentrations as compared to the control. Results obtained during the B-estradiol experiment. (n = 24)

control = 0 ethanol concentration

400 uM = 400 micromolar ethanol concentration

800 uM = 800 micromolar ethanol concentration

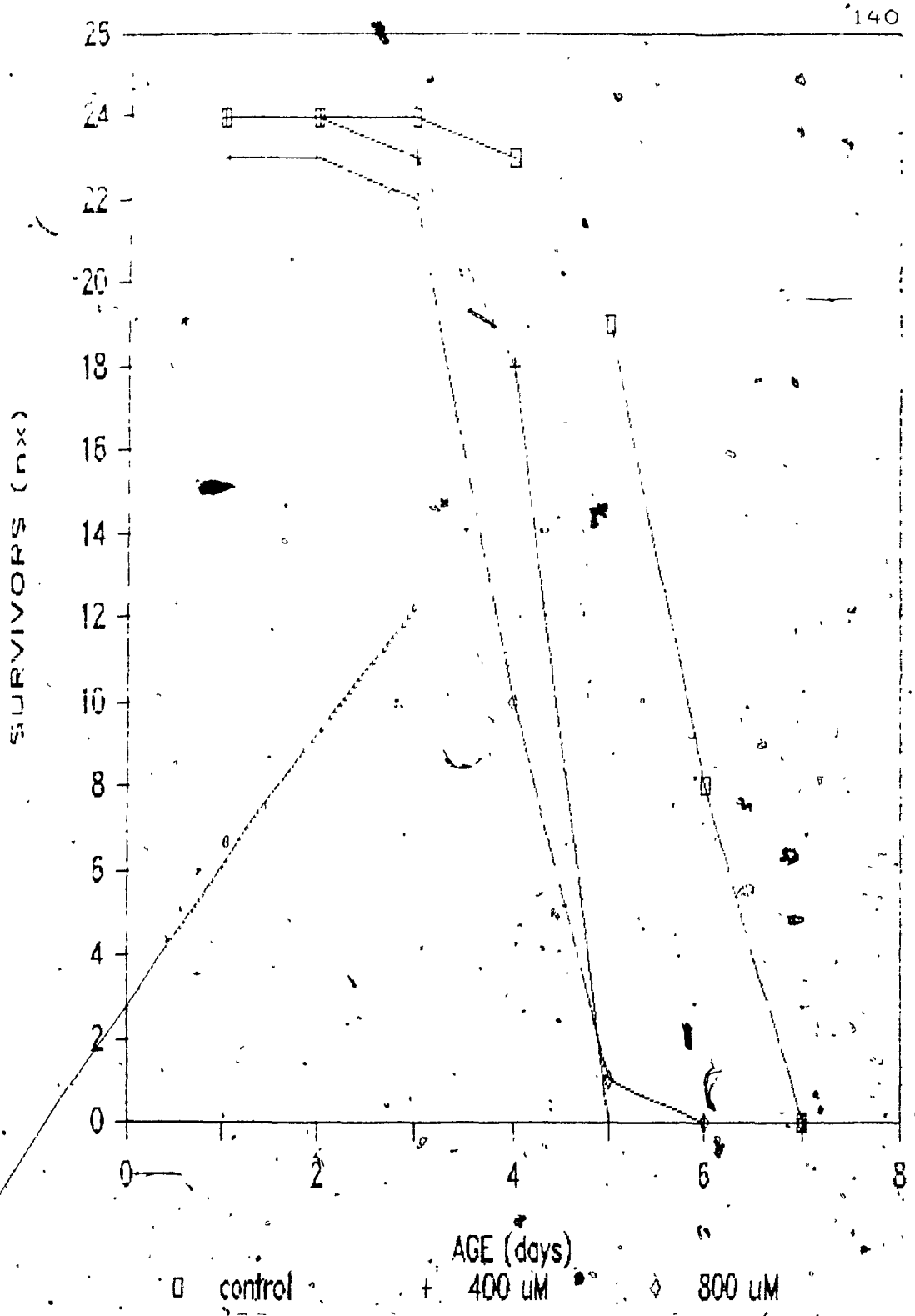

25
24
22
20
18
16
14
12
10
8
6
4
2
0
SURVIVORS (n×)
0
2
4
6
8
AGE (days)
control
400 uM
800 uM

Figure 32. Survivorship curves of A. brightwelli exposed to the higher range of ethanol concentrations as compared to the control. Results obtained during the thyroxine experiment. (n = 24)

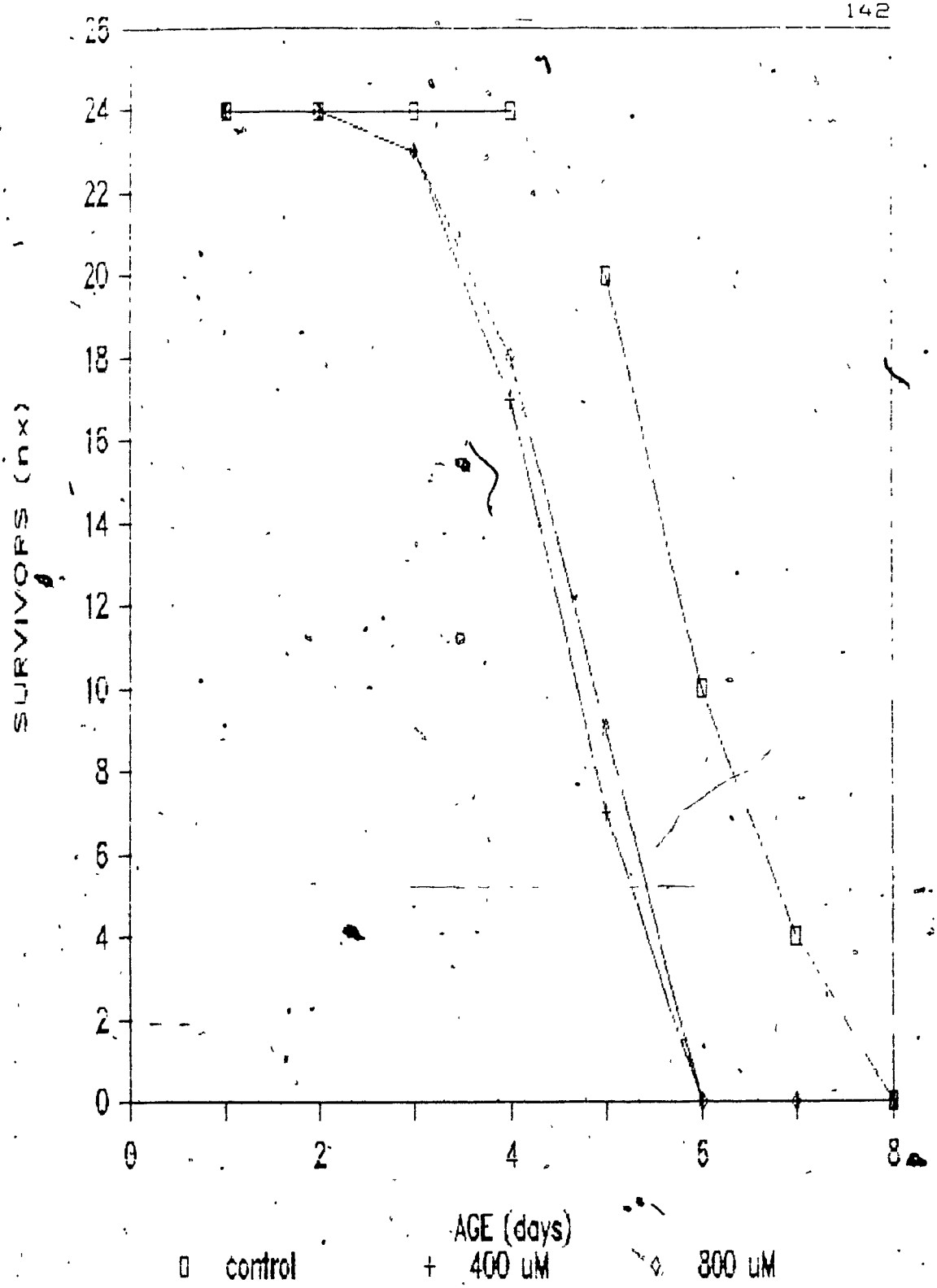

SURVIVORS (n x)
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
AGE (days)
control
400 uM
800 uM

Figure 34. Survivorship curves of A. brightwelli exposed to the higher range of ethanol concentrations as compared to the control. Results obtained during the cortisone experiment. (n = 24)

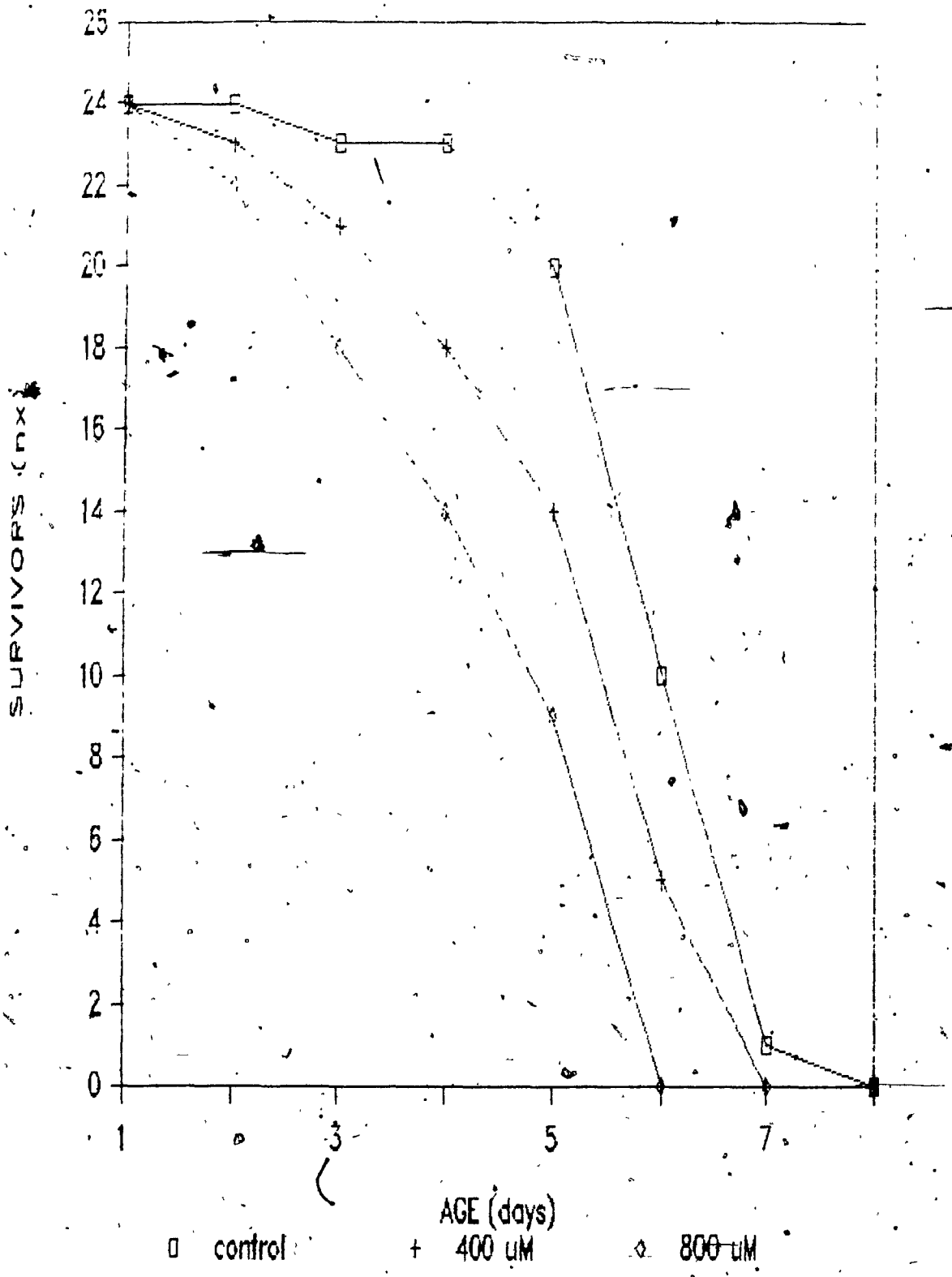

SURVIVORS (n x)
25
24
22
20
18
16
14
12
10
8
6
4
2
0
1
3
5
7
AGE (days)
control
400 uM
800 uM

Figure 36. Survivorship curves of A. brightwelli exposed to the higher range of ethanol concentrations as compared to the control. Results obtained during the hydrocortisone experiment. (n = 24)

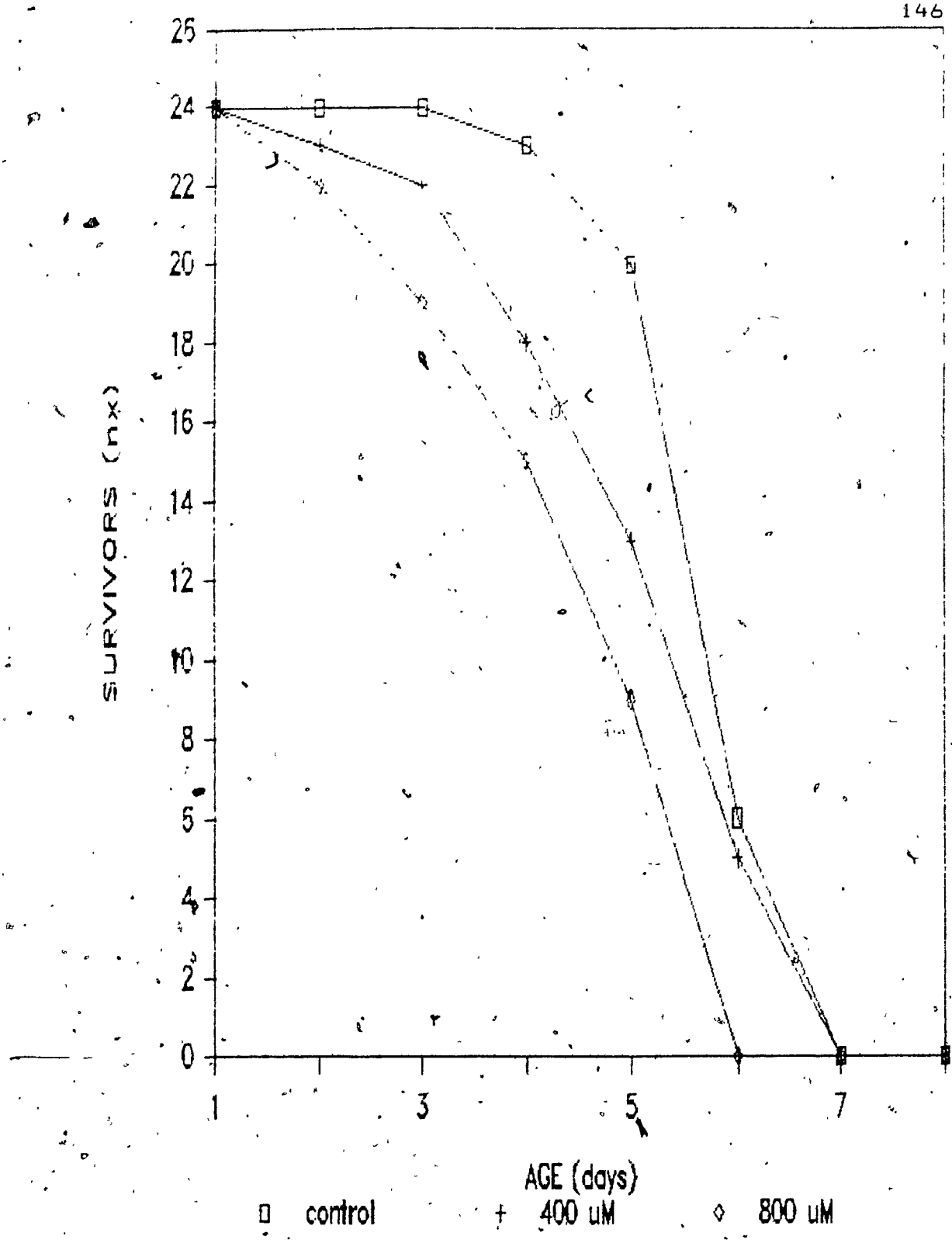

SURVIVORS (nx)
26
24
22
20
18
16
14
12
10
8
5
4
2
0
1
3
5
7
AGE (days)
control
400 uM
800 uM

Table 27

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT ETHANOL CONCENTRATIONS (B-estradiol experiment)

Ave. Concentr. (uM)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Offspring Number/ Rotifer
0	2.71 ± 0.08	2.21 ± 0.14	0.16 ± 0.08	5.83 ± 0.60
10	2.54 ± 0.14	2.54 ± 0.43	0	6.17 ± 0.95
50	2.92 ± 0.20	2.50 ± 0.42	0	5.33 ± 0.88
100	2.83 ± 0.14	2.25 ± 0.19	0	5.33 ± 0.58
200	2.67 ± 0.14	2.33 ± 0.13	0	5.17 ± 0.60
400	2.71 ± 0.11	1.46 ± 0.21	0	3.67 ± 0.62*
800	2.58 ± 0.47	1.30 ± 0.40	0	3.17 ± 0.41*

* Statistically significantly different from the control.

Table 28

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT ETHANOL CONCENTRATIONS
(thyroxine experiment)

Concentr. (uM)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Ave. Offspring Number/ Rotifer
0	2.88 ± 0.13	1.92 ± 0.51	0.91 ± 0.43	4.83 ± 0.42
10	2.48 ± 0.10	2.29 ± 0.14	0.64 ± 0.24	5.81 ± 0.57
50	2.39 ± 0.13	2.07 ± 0.19	0.79 ± 0.21	5.50 ± 0.44
100	2.42 ± 0.23	2.03 ± 0.27	0.84 ± 0.10	5.14 ± 0.48
200	2.28 ± 0.11	2.16 ± 0.17	0.56 ± 0.19	4.79 ± 0.59
400	2.21 ± 0.27	1.60 ± 0.21	0.94 ± 0.27	3.88 ± 0.62
800	2.18 ± 0.30	1.93 ± 0.30	0.72 ± 0.24	4.09 ± 0.49

- No significant difference between any group.

Table 29

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT ETHANOL CONCENTRATIONS
(cortisone experiment)

Concentr. (uM)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Ave. Offspring Number/ Rotifer
0	2.63 ± 0.17	2.33 ± 0.19	0.42 ± 0.11	6.33 ± 0.48
10	2.61 ± 0.19	2.37 ± 0.36	0.39 ± 0.14	6.25 ± 0.57
50	2.58 ± 0.10	2.20 ± 0.29	0.56 ± 0.10	5.83 ± 0.62
100	2.74 ± 0.11	2.39 ± 0.27	0.32 ± 0.14	7.00 ± 0.65
200	2.47 ± 0.17	2.21 ± 0.19	0.61 ± 0.17	6.08 ± 0.49
400	2.39 ± 0.10	1.98 ± 0.17	0.67 ± 0.13	5.33 ± 0.67
800	2.10 ± 0.08	2.11 ± 0.21	0.74 ± 0.20	4.05 ± 0.79

- No significant difference between any group.

Table 30

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI EXPOSED TO DIFFERENT EHTANOL CONCENTRATIONS (hydrocortisone experiment)

Concentr. (uM)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproduct. Time ± S.E. (days)	Postreprod. Time ± S.E. (days)	Offspring Number/ Rotifer
0	2.53 ± 0.07	2.63 ± 0.25	0.38 ± 0.08	8.25 ± 0.54
10	2.89 ± 0.08	2.15 ± 0.30	0.45 ± 0.09	8.00 ± 0.65
50	2.72 ± 0.10	2.29 ± 0.24	0.39 ± 0.10	7.89 ± 0.78
100	2.68 ± 0.11	2.17 ± 0.18	0.51 ± 0.08	7.08 ± 0.57
200	2.59 ± 0.13	2.34 ± 0.21	0.37 ± 0.11	7.38 ± 0.69
400	2.40 ± 0.09	2.03 ± 0.17	0.57 ± 0.07	7.00 ± 0.62
800	2.09 ± 0.13	2.19 ± 0.19	0.60 ± 0.14	6.88 ± 0.72

- No significant difference between any group.

Discussion

This discussion will serve to explain how nerve blockers and hormones affect rotifers. The results of all experiments will be discussed in detail in terms of rotifer lifespan and overall aging process. Special emphasis will be placed on any physiological changes occuring in the rotifer due to neuronal and hormonal influences. Some of the current theories of aging will be taken into account to explain some of the results.

The Nerve Blockers

The results presented in this thesis show that the nicotinic nerve blockers nicotine and procaine even at high concentrations did not affect rotifer lifespan or reproduction, but that the muscarinic nerve blocker atropine caused a significant increase in the lifespan of A. brightwelli. A possible explanation for these findings is that rotifers have muscarinic acetylcholine receptors, but not nicotinic receptors.

This idea of rotifers only having muscarinic

receptors is supported by other studies. Beauvais and Enesco (1985) found that low concentrations of the muscarinic nerve blocker curare increased rotifer lifespan and decreased the activity level of the rotifers. Thus curare and atropine, which are both muscarinic nerve blockers, both significantly extend lifespan in rotifers.

The only stage of the reproductive profile that is lengthened by atropine is the prereproductive stage. Thus the increase in the mean lifespan of the rotifer, induced by atropine, is due to a longer prereproductive period.

Beauvais and Enesco (1985) report that curare increases lifespan by extending the length of the prereproductive period. Thus the two muscarinic blockers curare and atropine act on the same phase of the lifespan of the rotifer.

These results cannot be compared to any other experiments done, because this line of investigation is quite new. There are no other studies of the effects of atropine on lifespan. The only paper that mentions any effect of atropine on some form of development is by Thomas, Esteves, Angluster, de Souza and Jurkiewicz (1981). These researchers added atropine to the

protozoan Herpetomonas samuelpessoai and began a specific differentiation process. Since the protozoans have a specific response to atropine, this suggests that the molecules for nerve trasnsmitters must have originated very early in evolution. Whereas atropine speeds up development in the protozoan, this nerve blocker slows down development into the adult stage of A. brightwelli by prolonging the prereproductive period.

To examine if the increase in rotifer lifespan observed with atropine treatment could be linked to a decrease in activity level, as is for curare, experiments were done to monitor the activity level of atropine-treated rotifers.

The results show that atropine does not affect the motor activity of the rotifer at any age. However, a note-worthy aspect of the results is that as the rotifer ages, its movement decreases. This age-related decrease in activity has also been observed in insects; the wing-beat frequency decreases in the house fly (Rockstein and Bhatnaga, 1966) and the mosquito has also shown a decline in flight performance with increasing age (Rowley and Graham, 1968).

This decrease in activity could be due to a

decrease in formation of acetylcholine, known to be present in the rotifer (Nogrady and Alai, 1983) and known to act as an excitatory neuro-transmitter at the neuromuscular junction. However, one cannot exclude the possibility of decreased efficiency of nerve transmission or weakening of muscle fibers that could occur in aging.

The results obtained in this activity study do not correlate with other research done in this area on invertebrates. Hillman, Ewert, Westerfield and Grim (1982) examined the influence of atropine on the motor activity of the nematode Brugia malayi. They found that atropine decreased motor activity by 71 %. This suggested to the researchers the possibility of a cholinergic nervous system being involved in the control of movement, yet effect on motility could be unrelated to cholinergic neurotransmission. In another experiment, Thomas, Esteves, Angluster, de Souza and Jurkiewicz (1981) observed when atropine was added to the protozoan Herpetomonas samuelpessoai, the cells became spherical and the movement of the protozoan was inhibited. Nogrady and Keshmirian (1986) observed the effect of atropine on the rotifer Brachionus calyciflorus. Normally when this rotifer swims, its long muscular foot is withdrawn into the lorica; it is only extended occasionally for a brief

moment. Addition of atropine resulted in the foot remaining extended; it stayed at a 90° angle to the body causing the foot to drag. It was assumed that atropine influenced the ion channels in the membrane. In all these examples, atropine decreases movement in some way and in the case of the nematode and rotifer it is believed to act by cholinergic neuromuscular mechanism.

These examples also suggest the presence of muscarinic receptors in the nematode and rotifer. It should be noted that only the foot of the rotifer Brachionus calyaflorus was paralyzed by atropine, other areas of muscular movement remained unaffected. The rotifer A. brightwelli used in the present study does not have a muscular foot. Thus it is possible that (muscarinic) receptors to which atropine will attach are not present at all neuromuscular junctions of the rotifer; this would explain why movement is not affected in A. brightwelli.

Since atropine did not influence lifespan through a change in activity level, another possibility was examined; that atropine might affect the food intake of the rotifer.

In this study, paramecium (food source for rotifers) stained with a biological stain called neutral

red were added with the appropriate atropine concentration to the rotifers. The goal was to examine the staining of the rotifer's gut; the amount of paramecium ingested determined the coloration of the rotifers gut. The darker the rotifer's gut, the more paramecium had been eaten. The results obtained for this experiment show that the control rotifers have bright red stomachs, whereas rotifers exposed to the atropine concentration that increased lifespan, have a pink stomach, indicating that these rotifer ate less; thus they underwent dietary restriction.

A number of investigators have shown that a decrease in food consumption results in a prolonged lifespan. Experiments done with rats (Berg and Simms, 1960; Reisen, Herbst, Walliker and Elvehjam, 1947), mice (Visscher, Ball, Barnes and Sivertsen, 1942), fish (Comfort, 1963), Daphnia (Ingle, Wood, and Banta, 1937), Drosophila (Loeb and Northrop, 1917) and rotifers (Fanestil and Barrows, 1965) show that this phenomena can be observed in many phyla; restriction of food from these organisms resulted in a significant prolongation of their lifespan. These studies confirm the first study done by McCay and his associates in 1935. They fed one set of young rats a restricted diet consisting of just enough food for normal development and fed a second set of rats

ad libitum. They found that rats maintained on a restricted diet lived longer than the rats fed ad libitum. The same phenomenon is observed in many species, as noted above.

To summarize, the results obtained for the nerve blockers show that nicotine and procaine, the nicotinic blockers, do not affect lifespan of A. brightwelli. In contrast. atropine, the muscarinic blocker significantly lengthens lifespan by increasing the prereproductive period. The lifespan experiment for each nerve blocker was repeated four times, obtaining the same results respectively for each blocker. This indicates that the results are highly reproducible.

One could conclude from the results that A. brightwelli does not have nicotinic receptors, but only muscarinic receptors, since this effect is only seen with atropine.

The increase in rotifer lifespan seems to be due to dietary restriction. The decrease in food intake could result in slower growth, leading to the prolongation of the prereproductive period.

It is possible that the atropine interferes with the coordinated movements of the cilia and digestive tract needed to engulf food. As noted earlier, atropine does not interfere with the normal rate of movement of

the entire animal. Nogrady and Keshmirian (1986) have also observed that atropine has different effects on different rotifer functions. These findings suggest the possibility that there are several types of nerve transmission in the rotifer.

If digestion and food intake is under neuronal control, than atropine could be acting as a blocker for the neurons which innervate the mastax and muscles of the viscera.

The Hormones

Thyroxine

Thyroxine does not affect the mean lifespan of the rotifer, even though a slight but insignificant decrease in lifespan does occur when rotifers are exposed to thyroxine at 400 and 800 uM concentrations. This slight decrease could be due to a mild toxic effect of thyroxine.

During the course of the discussion, when a hormone is described as having a toxic affect on the rotifer, what is meant is that the lifespan decrease caused by the

hormone is so extreme that this chemical is believed to be having a physiologically detrimental effect on the rotifer.

The reproductive period of rotifers exposed to 400 and 800 uM thyroxine is decreased significantly; the other stages of the lifespan are not affected. Offspring number is reduced at 400 and 800 uM thyroxine concentration. This is probably correlated with the decreased reproductive period; the rotifers exposed to these conditions had less time to produce offspring.

It is unlikely that thyroxine is influencing reproduction directly by reducing the reproductive period of the rotifer, since thyroxine is not a reproductive hormone. A simple explanation could be that thyroxine is slightly toxic at high concentrations and otherwise has no effect on rotifers.

No information is available on the effect of thyroxine on the lifespan of invertebrates. Loss of tissue responsiveness due to thyroxine may be involved in mammalian aging (Denkla, 1974). Thyroid implications in mammalian aging have been reviewed by Minaker et al, 1985.

B-Estradiol

The results obtained from this study show that as B-estradiol concentration increases, the mean lifespan of the rotifer decreases. The decrease is most pronounced at B-estradiol concentrations of 200, 400 and 800 uM.

The different stages of the overall rotifer lifespan are also affected by B-estradiol. The reproductive period is drastically shortened at concentrations of 200, 400 and 800 uM B-estradiol, and the postreproductive period at these concentrations is eliminated conmpletely.

This means that the decrease in lifespan of the rotifer is due to a shorter reproductive and postreproductive period. The offspring number at the different B-estradiol concentrations is affected as follows; at 100, 200 and 400 uM B-estradiol, the number of offspring is greatly reduced, and at 800 uM, no offspring are present. The decreased fertility is correlated with the reduced reproductive period.

The lifespan experiments were repeated four times and in each instance the results obtained were the same.

The decreased rotifer lifespan caused by B-estradiol could simply be a result of toxicity; B-

estradiol could simply be too toxic a chemical for the rotifers to survive. It is known that in mammals, such as rats, high levels of estrogen enhances aging of the hypothalamus in the brain and this hormone is also carcinogenic when present in excess (Zoler, 1983).

If estrogens have such extreme effects in mammals it might not be surprising that it has a toxic effect on the rotifer. Another explanation could be that reproduction in rotifers involves a reproductive hormone or compound that has similar properties to B-estradiol.

In this case, the externally added B-estradiol could have been recognized by receptors in the rotifer, thus influencing its physiology and altering its lifespan. It should be noted that the B-estradiol had no effect on the rotifer's prereproductive period; the rotifer was not influenced by the hormone until it started to reproduce. This shows that B-estradiol was acting during the reproductive period in the rotifer. The fact that it markedly reduces the number of offspring also supports the point of view that estrogen-like molecules are recognized by and act on the rotifer.

Invertebrates are not known to contain estrogens, but have other reproductive hormones that function in a similar manner. Crustaceans secrete a hormone from their

ovaries which control secondary sexual characteristics (Barrington, 1968). The corpora allata of insects releases a compound which influences egg development and the activity of the accessory reproductive glands (Barrington, 1968). Extensive research has been done on the effect of estrogens on mammals, but no documentation on whether estrogens influence invertebrates has been found. Thus no comparisons can be made between the influence of B- estradiol on the rotifer and its influence on other invertebrates.

Cortisone and Hydrocortisone

Cortisone and hydrocortisone both belong to a group of hormones called glucocorticoids. Glucocorticoids influence carbohydrate, fat, protein and purine metabolism (Hardy, 1981). Glucocorticoids are also known to inhibit inflammatory and allergic reactions in mammals by most possibly stabilizing lysosomal membranes which stops enzymes involved in inflammation from being released from lysosomes (Hadley, 1984).

Influence of Cortisone and Hydrocortisone on Life-span

The mean lifespan data shows that at cortisone concentrations of 100 and 200 uM, mean lifespan of the rotifer is significantly increased. Maximum lifespan increases by half a day at 100 uM cortisone with a value of 7.5 days as compared to 7.0 days for the control. The results obtained for hydrocortisone show that mean lifespan of the rotifer is increased at a hydrocortisone concentration of 100 uM. At hydrocortisone concentrations of 200, 400 and 800 uM mean lifespan drastically decreases.

The prereproductive and reproductive periods of the

reproductive profile increased at 200 uM cortisone concentration. Thus the overall increase in mean lifespan is due to the change in the prereproductive and reproductive period. At 100 uM cortisone, this trend is also observed, but the results do not reach statisitical significance. The stages of the reproductive profile were also affected by hydrocortisone. At 100 uM hydrocortisone, the prereproductive period was increased and the reproductive period was reduced. At 400 and 800 uM hydrocortisone, the prereproductive period was significantly shortened and the reproductive and postreproductive periods were both eliminated. The lifespan experiment was repeated four times for each glucocorticoid respectively. The results obtained were always the same as described above for both cortisone and hydrocortisone.

A comparison cannot be made between the results obtained in this study and any other previous studies, because research as to the effect of cortisone and hydrocortisone on the lifespan of vertebrates and invertebrates has not previously been investigated. The results show that cortisone and hydrocortisone both prolong rotifer lifespan by increasing the amount of time the organism spends in the developmental or juvenile

stage (prereproductive period). Thus cortisone and hydrocortisone could possibly be affecting growth rate and maturation by maintaining the rotifer in its early developmental stage for a longer time. In addition, hydrocortisone acts to reduce the reproductive time and delay sexual maturation.

The decrease in lifespan at the higher hydrocortisone concentrations could simply be because the hormone is becoming to toxic for the rotifer.

Fecundity

The offspring number of the rotifer is increased at cortisone concentrations of 100 and 200 uM. This effect is correlated with the fact that at these concentrations the reproductive period of the rotifer is increased allowing it more time to reproduce and increasing the number of offspring.

The offspring number of the rotifer is decreased at 100 uM hydrocortisone concentration. The lack of offspring at the three highest hydrocortisone concentrations is correlated with the absence of a reproductive phase and an overall short lifespan.

Influence of Cortisone and Hydrocortisone on Body Size

In order to examine the influence of cortisone and hydrocortisone on the rotifer, the body length and width of control rotifers and rotifers exposed to 10, 50, 100 and 200 uM cortisone concentrations and 10, 50, 100 uM hydrocortisone concentrations were measured.

Rotifers grow throughout their lifespan, they are at their smallest during the prereproductive stage.

The results obtained for body length and width show that rotifers exposed to 100 and 200 uM cortisone and to 100 uM hydrocortisone are always smaller than the control, no matter what age they are.

Experiments have not been done on the influence of cortisone on body size or weight in invertebrates, but Lau, Horowitz, Jumawan and Koldovsky (1978) examined the effects of cortisone on body weight and brain tissue growth in rat pups. They found that all cortisone-treated pups were smaller than their controls. Also, exposure to cortisone decreased the weight of the forebrain and cerebellum. The researchers could not explain why these changes occured.

It has been found that glucocorticoids inhibit growth in rapidly growing experimental mammals and children (Hadley, 1984). Investigators found that low

doses of glucocorticoids in liver, heart, skeletal muscle and kidney decreased DNA synthesis in the cells of these tissues and in this way caused a decrease in growth (Hadley, 1984). It is possible that hydrocortisone and cortisone could have a general effect on growth inhibition, explaining the decrease in size observed here.

Influence of Cortisone and Hydrocortisone on Lysosomes

Because of evidence that hydrocortisone and cortisone influence lysosomes (Guyton, 1981; Symons et al, 1969; Pollack and Brown, 1971) the presence of lysosomes in the rotifer were examined. A vital fluorescent stain (Fluoro-Bora) specific for lysosomes was used for this purpose.

One day to six day old cortisone-treated rotifers and control rotifers were examined for the presence of lysosomes. The stain did not allow for a quantative amount of lysosomes to be detected, instead the rotifers could only be observed as fluorescent (lysosomes stained) or none-fluorescent (lysosomes not stained). Since the lysosome-containing rotifers appeared as fluorescent cicrcular objects, it did not seem feasible to take pictures.

When the rotifers are one day old they do not fluoresce; this is true of the cortisone-treated rotifers and the control rotifers. From day 2 to day 6, the control rotifers and rotifers exposed to 10 and 50 uM cortisone all have a fluorescent appearance. At cortisone concentrations of 100 and 200 uM, the rotifers displayed no fluorescence at any age.

The results were the same for rotifers exposed to hydrocortisone. When the rotifers are one day old they do not fluoresce; this is true of the hydrocortisone-treated rotifers and the control rotifers. From day 2 to day 6, the control rotifers and rotifers exposed to 10 and 50 uM hydrocortisone all show fluorescence. Rotifers exposed to 100 uM hydrocortisone though do not fluoresce at any age. These results could be interpreted as follows:

Even if there is no fluorescence, lysosomes may still be present, in this case the lysosomes may either be to few or to small to absorb the stain as in the newborn rotifers, or the stain might not penetrate the lysosomal membrane.

If the membrane is stabilized by the glucocorticoids, the lack of staining could represent a failure of stain penetration in these conditions.

The mechanism of action of the FluoroBora stain is unclear. The staining system consists of highly boronic acid derivatives which are solubilized with a carrier buffer (Gallop et al, 1982). This system stains living cells and will transport water insoluble agents across the cell membrane. The system will then break up, allowing the fluorescent portion to enter lipophilic regions of the cell and the boronic acid will form complexes with hydroxyl and amino groups (Gallop, 1982).

Lysosomes are sac-like organelles consisting of a single lipoprotein membrane (Pitt, 1975; Dean, 1977). Lysosomes contain a large array of enzymes capable of degrading almost all of the important macromolecules of the cell (Pitt, 1975). Thus they play an important role in the destruction of foreign materials, thus they act as a defense mechanism for the organism (Dean, 1977). Release of of enzymes from lysosomes though can lead to injury or death of the cell and disease for the organism (Pitt, 1975).

Cortisone and hydrocortisone have been found to stabilize the membrane of lysosomes. Symons, Lewis and Ancill (1969) added cortisone to liver lysosomes of rabbits and found that less of the lysosomal enzymes acid phosphatase and B-glucuronidase were released from the

lysosomes, indicating that these were stabilized by cortisone. In another study by Pollock and Brown (1971), cortisone was also found to inhibit release of lysosomal enzymes in hepatic lysosomes.

It is possible that cortisone and hydrocortisone are stabilizing the lysosomal membranes in the rotifer, resulting in less degradative activity in the cells, thus slowing the aging process and resulting in the concurrent lifespan increase. It is possible that we do not observe the usual lysosomal staining in the cortisone and hydrocortisone treated rotifers because the cortisone and hydrocortisone stabilized the membranes to the extent that the dye can no longer penetrate and that cellular breakdown and turnover are reduced. This would explain why we see no stain in precisely those rotifers whose lifespan is significantly extended by cortisone or hydrocortisone treatment. Since the lysosome is a normal constituent of all eukaryotic cells, it is not reasonable to suggest that lack of fluorescent staining represents an absence of lysosomes.

Cortisone and hydrocortisone are both glucocorticoids and both increase the mean lifespan of the rotifer A. brightwelli. The lifespan increase in both cases is related to a decrease in growth rate and a

possible effect on lysosomes.

The rotifers might have receptors that recognize glucocorticoids which mediate the physiological changes reported here. However, no evidence of glucocorticoids in other invertebrates has been found. One instance where hydrocortisone has been implicated is in tissue culture studies of *in vitro* aging.

Cristofalo and Ragona (1982) report that addition of hydrocortisone to the culture medium delays senescence, allowing the cells to go through 25 to 50 % more population doublings. The mechanism of action by which hydrocortisone delays senescence and enhances population doubling in the tissue culture cells is not clear.

Ethanol

Ethanol was used as the solvent for all the hormones used in this study. During each hormone experiment, an ethanol control was run, which consisted of the same concentrations as the hormones, namely 10, 50, 100, 200, 400 and 800 uM. the results obtained from each of these runs will be briefly analyzed here.

Influence of Ethanol on Life-span

The results obtained for the mean lifespan of the rotifer indicate that ethanol does not affect the lifespan of the rotifer; only one set of results shows a decrease in lifespan at 400 and 800 uM, but the other three sets of results do not.

Ethanol was tested as a control at all these concentrations to make sure that the effects of the hormones on the rotifer were due to the hormones themselves and not due to the ethanol they were dissolved in.

From the results obtained, it is apparent that ethanol does not influence the lifespan of the rotifer. An excessive concentration of ethanol might have a toxic effect.

Fecundity

The offspring number was only reduced at 400 and 800 uM ethanol in one set of data. In all other cases, offspring number was not influenced by any of the ethanol concentrations.

Thus ethanol appears to have no effect on the fertility of the rotifer A. brightwelli.

Summary

Three nerve blockers, nicotine, procaine and atropine were examined for their effect on the lifespan and fecundity of the rotifer A. brightwelli. Nicotine and procaine did not influence the lifespan of the rotifer, whereas atropine increased rotifer lifespan.

The atropine concentration that caused a lifespan increase also produced a decline in food consumption of the rotifers as monitored by their intake of neutral red stained paramecium. The reason could be that digestion is under neuronal influence and atropine is inhibiting the feeding mechanism. Atropine is muscarinic nerve blocker.

Four hormones, thyroxine, B-estradiol, cortisone and hydrocortisone were examined for their effect on the lifespan and fecundity of the rotifer A. brightwelli.

Thyroxine had no effect on the rotifer, B-estradiol proved to be toxic, but both cortisone and hydrocortisone increased rotifer lifespan. Measurement of body size showed that less growth took place in the presence of specific cortisone and hydrocortisone concentrations. These glucocorticoids also altered the staining of

lysosomes, possibly by altering the permeability of the lysosomal membrane and decreasing cell turnover and degradation.

The lack of staining of lysosomes could be due to decreased membrane permeability which alters cell aging. However, the effect of the glucocorticoids on lysosomes in the rotifer is still speculative.

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Appendix I

Stock Buffer Solution

For culturing Paramecia/rotifers

COMPONENT	MOLECULAR WEIGHT	AMOUNT USED
distilled H_2O	–	250.0 ml
NaH_2PO_4	137.99	34.5 g
NaOH	40.0	4.0 g and appropriate amount of 0.5 M solution

Distilled water, NaH_2PO_4 and NaOH are added together in a 500 ml Erlenmeyer flask. In order to obtain a pH of 6.8, an appropriate amount of 0.5 M NaOH solution is added. For every liter of medium, 7.0 ml of the stock buffer solution is needed.

Appendix II

The following section contains the averages and F values of the replicate lifespan experiments for the hormones and nerve blockers.

Each replicate experiment for each chemical is referred to as a set, thus the first lifespan experiment done is called set 1, the second is set 2 and the third is set 3.

THE EFFECT OF NICOTINE ON THE LIFE-SPAN OF *A. BRIGHTWELLI* (N = 24).

	Concentration of Nicotine solution (percent)			
	0	0.000001	0.0000025	0.000005
Mean Lifespan ± S.E.M. (Days)				
Set 1	5.25 ± 0.21	5.29 ± 0.19	5.50 ± 0.27	5.32 ± 0.20
Set 2	5.32 ± 0.22	5.40 ± 0.17	5.65 ± 0.20	5.42 ± 0.29
Set 3	5.57 ± 0.30	5.34 ± 0.15	5.70 ± 0.29	5.57 ± 0.22

THE F VALUES OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN MEAN LIFE-SPAN OF NICOTINE-TREATED ROTIFER GROUPS WITH CONTROLS

Experimental Set	F value
Set 1	$F(3,92) = 0.678$; $p>0.05$
Set 2	$F(3,92) = 0.779$; $p>0.05$
Set 3	$F(3,92) = 0.694$; $p>0.05$

THE EFFECT OF PROCAINE ON THE LIFE-SPAN OF A. BRIGHTWELLI
(N = 24)

	Concentration of Procaine solution (E-05 %)						
	0	1	2.5	5	10	25	50
Mean Lifespan ± S.E.M. (Days)							
Set 1	5.43 ±0.15	5.40 ±0.13	5.47 ±0.21	5.39 ±0.16	5.45 ±0.22	5.37 ±0.14	5.10 ±0.21
Set 2	5.50 ±0.16	5.54 ±0.22	5.59 ±0.13	5.52 ±0.25	5.40 ±0.11	5.49 ±0.12	5.25 ±0.17
Set 3	5.37 ±0.11	5.39 ±0.25	5.45 ±0.13	5.49 ±0.19	5.36 ±0.24	5.31 ±0.22	5.08 ±0.16

THE F VALUES OF THE POST-HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN MEAN LIFE-SPAN OF PROCAINE-TREATED ROTIFER GROUPS WITH CONTROLS

Experimental Set	F value
Set 1	$F(6,161) = 2.374$; $p>0.05$
Set 2	$F(6,161) = 1.942$; $p>0.05$
Set 3	$F(6,161) = 2.748$; $p>0.05$

THE EFFECT OF ATROPINE ON THE LIFE-SPAN OF A. BRIGHTWELLI
(N = 24)

	Concentration of Atropine solution (percent)			
	0	0.0001	0.00025	0.0005
Mean Lifespan ± S.E.M. (Days)				
Set 1	5.65 ± 0.14	5.72 ± 0.10	6.15 ± 0.11	5.23 ± 0.21
Set 2	5.59 ± 0.08	5.63 ± 0.09	6.08 ± 0.13	5.11 ± 0.15
Set 3	5.69 ± 0.17	5.75 ± 0.14	6.13 ± 0.12	5.24 ± 0.19

THE F VALUES OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN MEAN LIFE-SPAN OF ATROPINE-TREATED ROTIFER GROUPS WITH CONTROLS

Experimental Set	F value
Set 1	$F(3, 92) = 4.784$; $p \leq 0.05$
Set 2	$F(3, 92) = 4.653$; $p \leq 0.05$
Set 3	$F(3,92) = 4.582$; $p \leq 0.05$

THE EFFECT OF THYROXINE ON THE LIFE-SPAN OF A. BRIGHTWELLI
(N = 24)

	Concentration of Thyroxine solution (uM)						
	0	10	50	100	200	400	800
Mean Lifespan ± S.E.M. (Days)							
Set 1	5.69 ±0.23	5.47 ±0.21	5.00 ±0.19	5.19 ±0.24	5.10 ±0.17	4.33 ±0.25	4.12 ±0.19
Set 2	5.70 ±0.29	5.64 ±0.14	5.35 ±0.21	5.24 ±0.25	5.17 ±0.19	4.10 ±0.23	4.06 ±0.27
Set 3	5.63 ±0.25	5.48 ±0.18	5.24 ±0.17	5.29 ±0.24	5.06 ±0.27	4.19 ±0.19	4.12 ±0.22

THE F VALUES OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN MEAN LIFE-SPAN OF THYROXINE-TREATED ROTIFER GROUPS WITH CONTROLS

Experimental Set	F value
Set 1	$F(6,161) = 2.134;\ p>0.05$
Set 2	$F(6,161) = 2.083;\ p>0.05$
Set 3	$F(6,161) = 1.998;\ p>0.05$

THE EFFECT OF B-ESTRADIOL ON THE LIFE-SPAN OF A. BRIGHTWELLI
(N = 24)

	Concentration of B-estradiol solution (uM)						
	0	10	50	100	200	400	800
Mean Lifespan ± S.E.M. (Days)							
Set 1	5.29 ±0.11	5.00 ±0.15	4.98 ±0.25	4.76 ±0.34	2.85 ±0.23	2.36 ±0.19	2.39 ±0.29
Set 2	5.17 ±0.21	4.99 ±0.24	4.85 ±0.11	4.62 ±0.19	2.92 ±0.17	2.43 ±0.27	2.40 ±0.26
Set 3	5.23 ±0.32	5.15 ±0.23	4.85 ±0.17	4.72 ±0.14	3.08 ±0.22	2.76 ±0.27	2:53 ±0.13

THE F VALUES OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN MEAN LIFE-SPAN OF B-ESTRADIOL-TREATED ROTIFER GROUPS WITH CONTROLS

Experimental Set	F value
Set 1	$F(6,161) = 10.135;\ p \leq 0.01$
Set 2	$F(6,161) = 11.098;\ p \leq 0.01$
Set 3	$F(6,161) = 10.736;\ p \leq 0.01$

THE EFFECT OF CORTISONE ON THE LIFE-SPAN OF A. BRIGHTWELLI
(N = 24)

	Concentration of Cortisone solution (uM)						
	0	10	50	100	200	400	800
Mean Lifespan ± S.E.M. (Days)							
Set 1	5.41 ±0.32	5.49 ±0.29	5.53 ±0.27	6.56 ±0.35	6.43 ±0.32	5.18 ±0.21	5.07 ±0.24
Set 2	5.50 ±0.24	5.61 ±0.23	5.65 ±0.19	6.62 ±0.14	6.54 ±0.32	5.23 ±0.21	5.10 ±0.22
Set 3	5.45 ±0.11	5.50 ±0.15	5.57 ±0.23	6.52 ±0.32	6.47 ±0.22	5.12 ±0.27	5.09 ±0.17

THE F VALUES OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN MEAN LIFE-SPAN OF CORTISONE-TREATED ROTIFER GROUPS WITH CONTROLS

Experimental Set	F value
Set 1	$F(6,161) = 6.988$; $p \leq 0.01$
Set 2	$F(6,161) = 7.082$; $p \leq 0.01$
Set 3	$F(6,161) = 7.237$; $p \leq 0.01$

THE EFFECT OF HYDROCORTISONE ON THE LIFE-SPAN OF
A. BRIGHTWELLI (N = 24)

	Concentration of Hydrocortisone solution (uM)						
	0	10	50	100	200	400	800
Mean Lifespan ± S.E.M. (Days)							
Set 1	5.57 ±0.11	5.59 ±0.21	5.43 ±0.17	6.38 ±0.19	2.19 ±0.23	0.93 ±0.13	0.84 ±0.17
Set 2	5.63 ±0.21	5.67 ±0.12	5.53 ±0.17	6.54 ±0.16	3.45 ±0.14	1.02 ±0.22	0.87 ±0.23
Set 3	5.51 ±0.11	5.62 ±0.14	5.59 ±0.09	6.37 ±0.08	1.84 ±0.21	0.88 ±0.10	0.69 ±0.12

THE F VALUES OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN MEAN LIFE-SPAN OF HYDROCORTISONE -TREATED ROTIFER GROUPS WITH CONTROLS

Experimental Set	F value
Set 1	F (6,161) = 10.345; $p \leq 0.01$
Set 2	F (6,161) = 11.032; $p \leq 0.01$
Set 3	F (6,161) = 10.473; $p \leq 0.01$

Appendix III

Photomicrographs

C. Reichert light microscope with a camera attachement was used to take the photomicrographs. Kodacolor VR color film; ASA 1000 was used.

PLATE 1. A two day old A. brightwelli exposed to regular medium (control). The medium contained paramecia stained with 0.75 ug/ml neutral red. The gut coloration of the rotifer is caused by ingestion of paramecia stained with neutral red.

PLATE 2. A two old A. brightwelli exposed to 0.0001% atropine solution. The solution contained parameciua stained with 0.75 ug/ml neutral red. The gut coloration is caused by ingestion of paramecia stained with neutral red.

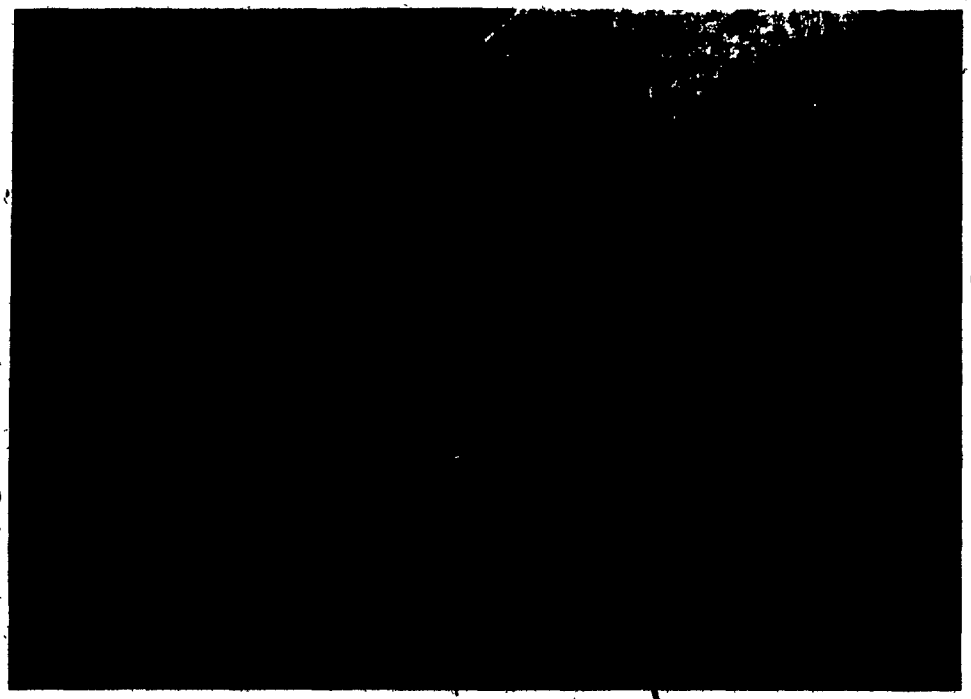

PLATE 3. A two day old A. brightwelli exposed to 0.00025% atropine solution. The solution contained paramecium stained with 0.75 ug/ml neutral red. The gut coloration of the rotifer is caused by ingestion of paramecia stained with neutral red.